THE UPTAKE AND EXTRACTION OF HEAVY METALS FROM CONTAMINATED SOIL BY COPPICE WOODLAND

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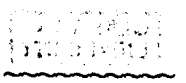

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Summary

Phytoremediation is a developing technology which has been highlighted as an effective and cheap method for cleaning up soil contaminated with industrial wastes which contain both organic and inorganic chemicals. The enhanced microbial activity within the rooting zone of plants is believed to promote the breakdown of organic contaminants to carbon dioxide and water. The remediation of heavy metals requires the accumulation of heavy metals within the tissues of the growing vegetation. When the biomass is harvested metals held within the tissues are removed from the site.

Trees have been identified as suitable phytoremediation species; they are long lived, have high biomass production rates and are commonly found growing on derelict and contaminated sites which indicates that they are suited to such environments. Interest has also been raised with respect to the use of coppice woodland to produce renewable fuels for energy production.

The research undertaken was designed to investigate the potential use of coppice woodland for the clean up and remediation of heavy metal contaminated soil. Particular attention was given to high yielding coppice woodland species, especially willow and poplar. This was because breeding and field trials have reported that some hybrid willow clones can produce biomass yields of up to 60 t ha^{-1} y^{-1}.

The experimental works undertaken comprised three experiments which have been referred to as the 'Field Studies', 'Pot Studies' and 'Hydroponic Studies' and describes the medium in which the trees were grown and studied. The experiments were devised to study the variation in the uptake of metals between different tree species growing in different environments.

The findings of the studies generally indicate that metal uptake rates determined in the field were lower than the uptake levels recorded in the pot and hydroponic studies. These differences were attributed to the increased availability of the study

metals in the pot and hydroponic studies. Zinc concentrations in the above ground tissue of willow determined from the three experiments ranged from 159 to 223 mg kg^{-1} in the field study, 281 to 2995mg kg^{-1} in the pot study and 40 to 5530 mg kg^{-1} in the hydroponic study.

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Zinc was the only metal accumulated to significant concentration within the biomass of field samples. Zinc, copper, cadmium, nickel and chromium were accumulated in the biomass of seedlings grown in the pot studies and zinc, copper, cadmium, nickel, chromium and to a lesser extent lead were accumulated by some of the hydroponic study trees. Some of the uptake levels recorded were not dissimilar from accumulation levels reported in hyperaccumulater species and highlights the potential of some tree species to accumulate metals in above ground tissues (leaves, twigs and stem).

The pot and hydroponic studies suffered high seedling fatality rates which were attributed to metal toxicity and/or salinity. These findings could indicate possible establishment problems when trying to plant trees on contaminated sites.

The feasibility of using coppice woodland to clean up zinc contaminated soil was assessed with regard to the maximum uptake levels of zinc determined by the experimental works. Provisional remediation time scales may be in the order of between 80 and >800 years. However, actual time scales will depend on the metal, its availability and the starting concentration of the metal in the soil. It unlikely such long timescales would be acceptable to developers.

However, coppice woodland can offer a number of additional benefits including habitat creation, reduction in soil erosion and screening unsightly sites. Future developments in plant breeding and genetic engineering are currently underway to improve yields and increase metal accumulation. Such future developments should reduce remediation time scales.

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INTRODUCTION

1.1 INTRODUCTION

The funding for this graduate research scholarship was awarded to the University of Glasgow by Scottish Enterprise, Department of Environmental Development. The research work was conducted at the University of Glasgow within the Departments of Environmental Chemistry and Botany.

The aim of the research was to identify a cost effective and reliable remediation technique for the clean-up of heavy metal contaminated soil. The research comprised investigative, experimental and analytical studies to identify suitable coppice woodland tree species which could be used to remediate heavy metal contaminated soil. The research work was undertaken by Scott D McGregor under the supervision of Dr. H Duncan and Dr. I Pulford, Department of Environmental Chemistry and Dr. C Wheeler, Department of Botany.

The scope of the study comprised the following elements;

- Background information, including the requirement for site remediation works to remove heavy metals from soil
- A literature review to identify the work of previous researchers on the uptake of heavy metals by vegetation and the behaviour of heavy metals in soil.
- The design and implementation of experimental works to assess the growth and metal uptake of selected tree species. The works implemented fall within 3 main experiments; the study of natural woodland populations growing on derelict land; the study of selected tree seedlings planted in artificially contaminated soil; and the study of selected tree seedlings planted in nutrient solutions containing heavy metals.
- Reporting the findings of the experimental work
- An appraisal of the findings with regard to the use of coppice woodland for the clean up of contaminated land.

1.2 BACKGROUND

1.2.1 Derelict Land

The economic significance of derelict and contaminated land is immense. Records indicate that in 1988 there were approximately 40,500 hectares of derelict land in England and Wales (Department of the Environment, 1991a) and 7400 hectares in Scotland (Scottish Office, 1990). These figures do not take into account land used for mineral extraction.

Derelict land is often non-productive and in some instances can present a variety of hazards. The main hazards are from materials which are present in the soils and include chemical contaminants, explosive materials and combustible materials Derelict land is also ugly and unsightly and its very nature detracts from the image of the surrounding area.

The remediation of any derelict site presents a number of potential hazards to the developer, which have to be overcome prior to redevelopment; this is particularly important where potentially hazardous or toxic chemicals exist in the soil. In such instances the redevelopment of sites will require some level of remediation to remove or reduce the concentration of hazardous materials to a safe level.

The range of chemicals which may be present at a site can include organic and inorganic contaminants which have generally arisen from former industrial operations at the site. Inorganic heavy metals are one such group of contaminants which can be toxic to a wide variety of organisms including soil microbes, plants, animals and humans. Although natural soils can contain elevated concentrations of some heavy metals, the recent trend towards the redevelopment of brown field sites has highlighted the problems associated with elevated concentrations of heavy metals which require treatment before construction begins. Many former industrial activities including iron and steel works, shipbuilding, railways and gas production were responsible for generating significant volumes of waste. Some of these wastes

contain elevated concentrations of heavy metals and other contaminants at concentrations which are a hazard to human health. At the time these industries did not recognize the contaminating potential of the waste and little or no control was implemented to contain the waste and prevent the spread of contamination. This has resulted in a legacy of contaminated land which has to be remediated prior to redevelopment.

These areas of contaminated land are often located within cities and towns which had originally developed around the industries which caused the contamination. Today with greater restrictions on out of town developments and the protection of green belts greater emphasis is being placed on the redevelopment of derelict land to housing and light industrial developments. Therefore there is an increasing need for processes which can remediate contaminated soil.

It should be noted that no treatment exists for the destruction of heavy metals in soil. At present the most common procedure used by the construction industry and developers for removing heavy metal contamination is excavation and removal to landfill. Other less favoured options include: encapsulation of contaminated material below an effective depth of clean material; adding chemical reagents which fix the metals within the soil matrix; and adding chemicals which change the metal to a less toxic species in the soil including the formation insoluble metal precipitates.

1.2.2 Phytoremediation

The uptake of heavy metals has long been recognised as a feature of vegetation growing on contaminated land and site remediation works often specify measures to protect the vegetation in landscape planting (ICRCL, 1987). Therefore any plant which can remove metal from the soil and accumulate it in the above ground biomass can reduce the metal concentration in the soil. The process of metal removal by plants is termed phytoremediation or phytoextraction. Phytoremediation relies on the removal of heavy metals from the soil by the process of plant uptake

and accumulation in the above ground components of the plant. When the plant is harvested the metal present within the plant is removed from the site.

In the United States contaminated land is regarded as a liability and billions of dollars are spent each year on the remediation of contaminated soils. Phytoremediation exploits the ability of plants to influence the biological, microbial, chemical and physical processes to remediate soil. Phytoremediation also offers economic, aesthetic and technical advantages when compared with traditional engineering solutions (Cunningham & Berti, 1993).

The need for developing clean-up technology has resulted from uncertainty in environmental regulation, different efficiencies of remediation and the absence of clean-up technology for several major pollutants. The benefits of trees for use in phytoremediation include: extensive root systems; the support of a large diversity of microorganisms; growth on land of marginal quality; lowest cost plant type and their role as effective barriers to the movement of contaminated groundwater. In addition the 'crop' does not enter the human food chain and with coppice woodland new shoots generate from the cut stem alleviating the need to replant (Stomp et al., 1994).

For the effective phytoremediation of heavy metal contaminated soils it is important to give careful consideration to optimising the uptake capacity and yield of the chosen vegetation used. Other considerations are ease of maintenance, ease of harvest and the commercial value of the crop. Rooting density is important and vegetation which has a significant root distribution in the soil will be more effective at removing metal.

It has been identified that certain plants can accumulate both essential and non-essential heavy metals in roots, shoots and leaves to concentrations in excess of those in the soil. The process is often termed bioconcentration of heavy metals in plants and Raskin et al., (1994) suggests the two basic strategies used by plants to tolerate heavy metals are:

- Metal Excluders exclude metals but have still been shown to accumulate high metal concentrations in the roots
- Metal Non-excluders actively accumulate metal in above ground tissue and can be divided into indicators (metal biomass concentrations reflect concentrations in the soil) and hyperaccumulators (accumulate metal to concentrations higher than in the soil)

Hyperaccumulator plants already identified include *Brassicaceae* (mustard family) and *Euphorbiaceace.* The Caledonian tree (*Sebertia acuminata*) contains up to 11% nickel in the latex and *Armeria martima* can accumulate lead to 1% in biomass. These findings indicate the potential for phytoremediation to be developed as an effective clean-up technology (Raskin et al., 1994).

1.2.3 Coppice Woodland

Coppice woodland is a historical method of woodland management. Following harvest new shoots are generated from the cut stem of a harvested tree. In recent years European countries including Sweden and the United Kingdom have undertaken field trials to evaluate the commercial benefit of coppice woodland for the production of biomass for electricity generation (Piper, 1994).

The trials have tended to concentrate on the use of hybrid poplar and willow clones which have been selected primarily for biomass production. The results of some field trials have reported that some willow clones can produce up to 35 tons of biomass per ha per year when growing under favourable conditions. It should be noted that these yields would be very unlikely for identical species planted on contaminated and derelict land.

1.3 LITERATURE REVIEW

This literature review was undertaken to access the extent of previous work and was targeted towards a review of research papers and other published information relating to the uptake of heavy metals by vegetation. Relevant subject papers were identified from key word searches of both the chemical abstract and biological data bases. The papers were sourced from journals held by the University of Glasgow and inter-library loan requests.

The literature review identified a variety of studies which had investigated the uptake of heavy metals from contaminated soil. However, no previous research had studied the uptake of heavy metals from derelict and contaminated land by short rotation coppice woodland as a means of phytoremediation. The previous studies which contained information on the uptake and biomass concentration of heavy metals had been primarily undertaken to address other areas of research and environmental concern. The main topics of previous research are listed below and will be discussed in more detail under the relevant headings.

- air pollution
- metal smelting operations
- mine tailings
- soil acidity
- nutrient studies
- human health implications
- environmental dating
- metal movement in vegetation
- metal resistance studies
- root mycorrhizae and soil microorganisms
- establishment of vegetation on derelict land
- metal uptake
- energy generation
- hyperaccumulation

The literature review was not solely limited to a review of heavy metal uptake by vegetation. The importance of metal behaviour in soil has also been recognised with regards to phytoremediation. In this regard the theory of metal retention, mobility and transformation in the soil environment will be discussed in Section 1.4.

1.3.1 Air Pollution

Many industries worldwide emit contaminants into the atmosphere. Historically the burning of fossil fuels released significant quantities of contaminants including heavy metals, particulates, sulphur dioxide and oxides of nitrogen to the atmosphere. In more recent times motor vehicles, incinerators and modern industrial processes have continued to release lead, oxides of nitrogen and sulphur, volatile organic compounds and dioxins, to name but a few, to atmosphere. Government legislation including the 'Clean Air Acts' and a greater awareness of the environment, combined with increased monitoring and regulatory control has led to a reduction in the emissions of many air pollutants in gaseous and particulate forms.

The presence of some common industrial activities have been shown to influence metal contents in vegetation. Leaves of birch trees collected from various locations were analysed for a number of chemical elements (Cr, Ni, Zn, Cd and Pb). Hoffmann & Lieser (1987) reported that railway lines were a source of chromium and zinc, industrial plants were a source of zinc and cadmium and automotive traffic was a source of lead.

Woody plants are reported to play an important role in heavy metal recycling in urban environments by acting as short and long term storage sinks. Lead associated with foliage can be returned to the soil or atmosphere in a relatively short time scale while lead held in twigs and other perennial tissue may be removed from circulation for extended periods of time. Branch samples collected in the city of New Haven, USA during the period September to November 1970 contained average lead

concentrations of 478 ppm while the lead foliage concentrations in unpolluted environments was 1 ppm. The highest lead concentrations were in the twigs of sugar maple, eastern hemlock, yew and Norway spruce to a maximum of 760 mg kg^{-1} dry weight. Washing the foliage samples prior to analysis was found to remove a small quantity of lead but most was found to remain within the leaf (Smith, 1972).

In urban environments trees have sometimes been regarded as biological filter systems which filter out harmful air pollutants. This ability of trees to filter out dusts and industrial gases is reported to have been used to beneficial effect in Poland where trees are planted in thick green belts around industrial areas to reduce the effects of air pollution. In studies carried out deciduous trees were found to be good absorbents of such pollution (Idzikowska, 1983).

It is not clear what procedure trees use to filter out harmful air pollutants but in one study the leaves of study trees accumulated between 1.2 and 9 times more metal when growing in an industrial environment as compared with identical tree species growing in rural environments (Kovacs et al., 1981).

Concerns over the effects of industrial emissions on the growth of European forests have promoted a number of studies. Burton et al. (1983) determined the critical concentration of several heavy metals in Sitka spruce by measuring the concentration of heavy metals in the foliage of Sitka spruce growing in South Wales. The upper critical concentrations which were detrimental to tree growth were reported as 4.8 mg kg^{-1} cadmium, 5.8 mg kg^{-1} nickel, 19 mg kg^{-1} lead, 88 mg kg^{-1} copper and 226 mg kg^{-1} zinc. The study concluded that the levels of nickel and cadmium in industrial emissions might be present at such concentrations in some areas that the pollution might affect the normal functioning and growth of some forests. The levels of lead, copper and zinc were considered to pose no risk.

In another study of air pollution the needles of Norway Spruce trees growing in Switzerland were analysed for a number of elemental air pollutants. The study also tried to investigate the distribution of different elements in and on the foliage.

Landolt et al., (1989) concluded that needle analysis was a valid instrument for the identification of various air pollutants and that the correlation between air pollutants and levels in needles was best for lead and decreased in order of molybdenum, iron, cadmium and sulphur.

From previous studies it is evident that tree foliage has the ability to accumulate atmospheric pollutants including heavy metals. This is especially true for trees growing within industrial environments. The accumulation of heavy metals as a result of air pollution is thought to be predominantly due to the process of deposition and adsorption on the external surfaces of leaves, needles and bark. The uptake of metal from the soil in such situations is considered to be of secondary importance. In this regard studies which discuss the findings and effects of air pollution are considered to offer little information on the uptake of metal contaminants from the soil.

1.3.2 Metal Smelting Operations

Smelting industries are known to emit significant quantities of air borne contaminants including sulphur oxides and particulate material which often contain elevated concentrations of zinc, lead, cadmium, copper, nickel and chromium (Rachwal et al., 1992). This 'fallout' of air borne contaminants was found to cause the accumulation of heavy metals in the surface layers of the soil in close proximity and down wind of smelters.

Little & Martin (1972) analysed foliage and soil near a smelting complex and reported the distribution of air borne zinc, lead and cadmium was strongly influenced by prevailing wind conditions. Zinc, lead and cadmium were detected in elm leaves to maximum concentrations of 8000, 5000 and 50 mg kg^{-1} dry weight, respectively, at distances of between 10 and 15 kilometers from the point of emission.

In addition to the metals, gases of sulphur and nitrogen oxides are emitted. These gases dissolve in water to form acidic solutions which can cause localized soil acidification. Such acidification is known to increase the solubility and mobility of metals in soils which can increase the availability of some metals to vegetation. Lobersli & Steinnes (1988) found manganese, magnesium and calcium concentrations in soils increased with distance from the smelter. In addition decreased concentrations of manganese, magnesium and calcium were recorded in vegetation growing near smelter sites. These observations were attributed to a decrease in base saturation of the soil caused by the increased leaching of these metals from the soil. The studies also observed decreasing concentrations of copper, zinc and lead in foliage with distance from the smelter in all species analysed and was attributed to dilution. This effect of the elevated heavy metal concentrations combined with increasing soil acidity has a severe detrimental effect on vegetation growing in close proximity to smelters. In such cases vegetation is destroyed or seriously injured and re-vegetation is difficult.

Increasing levels of air pollution from smelting operations have been reported by Hawrys (1984) to cause mortality of tree seedlings, height reductions of between 28 and 48% and shortening of the foliation period by two weeks. The levels of zinc, lead and cadmium were recorded to increase in leaves proportionally to the degree of air pollution. Maximum foliage metal concentrations of 1000 mg kg^{-1} zinc, 30 mg kg^{-1} lead and 4 mg kg^{-1} cadmium were recorded in *Populus tremula* leaves.

Similar investigations at a copper/zinc smelter site where operations had been ongoing for more than 50 years recorded high levels of zinc, copper and lead in surface soils down wind of the site (Hogan & Wotton, 1984). The effects of these smelter emissions included increased tree mortality, reduction in species diversity, reduced growth in surviving species and increased soil erosion. Metal concentrations were highest at the soil surface from the aerial deposition of metal particulates. The most impacted soils contained in excess of 1000 mg kg^{-1} copper and 4000 mg kg^{-1} zinc, however, no evidence of soil acidification was noted. Metal

availability was assessed at between 50 and 60% of total zinc and between 18 and 36% of total copper.

Analysis of foliage collected from the most abundant trees and shrubs growing at the site identified elevated levels of zinc, copper and lead in the foliage of the species analysed. The results are summarised:

Species	Zinc	Copper	Lead
Jack Pine	75 - 506	3 - 33	5 - 62
Alder	53 - 473	9 - 32	7 - 38
Black Spruce	62 - 455	2 - 18	5 - 31
Labrador lea	64 - 376	7 - 35	6 - 34

All values are expressed as mg kg^{-1}.

It should be noted that no attempt was made to distinguish between the internal and external concentrations of metal on the foliage.

In an attempt to study the effects of copper smelter emissions, cuttings of poplar clones were planted in a substrate of sand and heavy metal dust collected from the electrofilters of a smelter (Rachwal et al., 1992). The three substrate mixtures comprised 1, 5 & 25% smelter dust in sand. Tolerant species of poplar were found to accumulate higher concentrations of most heavy metals except for Cd and Ni. The maximum concentration of each metal analysed in study trees in mg kg^{-1} dry weight are given below:

Metal	Pb	Cu	Zn	Fe	Cd	Mn	Cr	Ni	Co
Concentration	124.5	506.1	210.7	263.5	1.7	42.4	2.4	3.4	3.4

Tyler (1984) reported that the long term effects on a coniferous forest soil of copper and zinc smelter emissions were that heavy metal toxicity was more pronounced in the organic layer of topsoil (mor horizon). Mosses and lichens were killed and the growth of vascular plants was reduced. Analysis of the organic horizon recorded

maximum total zinc, copper and lead concentrations of 10000, 20000, and 500 mg kg^{-1} respectively with soil pH levels of between 4 and 6.

Mejnartowicz (1986) studied the influence of copper smelter emissions on alders growing within the zone of emission. The trees on average contained 200 times more copper and 3 times more zinc than similar trees growing in unpolluted environments. The concentrations recorded in biomass varied between 1000 and 1500 mg kg^{-1} copper, 200 and 400 mg kg^{-1} zinc and 400 and 2000 mg kg^{-1} manganese. It was suggested that copper and zinc accumulated in shoots over the winter period and were mobilised and transferred to developing leaves during foliation.

In another study soil and vegetation samples were collected along a 12 kilometer transect near a nickel/copper smelter at Coniston, Ontario, Canada (Hazlett et al., 1983). The soils were found to contain up to 1.2, 0.97 and 23.0 % of nickel, copper and iron respectively and the soil was acidic (pH 2.4), however, manganese and zinc were at normal soil concentrations. The concentrations of heavy metals in foliage samples were erratic with distance from the smelter and nickel was the only element generally present at elevated concentrations in vegetation.

One of the major areas of study in regions affected by smelter emissions is the revegetation of areas were the native plants have been destroyed. Rachwal (1983) set up experimental plots containing 270 different species at distances between 800 and 1400 meters down wind of a smelter. During the experimental period (1973 to 1976) the study species were observed for % leaf injury, survival, vitality, premature defoliation and growth. Only a few of the study species were found to be tolerant of the prevailing conditions at 800 meters. However, a number of medium resistant species were found to be suitable for re-cultivation at greater distances from the source of the emission. *Populus x canadensis 'Marilandica'* was found to be relatively tolerant to increasing metal contamination and analysis results showed a tendency for the species to accumulate some heavy metals, although not cadmium and nickel. The recorded metal concentrations (mg kg^{-1} dry weight) were as follows:

P. x canadensis	Pb	Cu	Zn	Fe	Cd	Mn	Al	Cr	Ni	Co
'Marilandica'	125	506	215	264	1.6	42	266	2.4	3.3	3.4

The effects of metalliferous dust from copper smelters has been reported to cause morphological and ultra-structural changes in trees (Wozny *et al*, 1992). The toxic influence of dust constituents were studied on selected poplar hybrids (*Populus x canadensis* '*Marilandica*' and *Populus x NE42*). The studies found the effects were most marked in the root hair region which was more easily damaged in the susceptible hybrid. The more tolerant poplar (*Populus x canadensis 'Marilandica'*) took up more lead, zinc and copper.

The germination of seeds is recognised as an important factor in the recovery and re-colonisation of natural woodlands which have been destroyed by smelter emissions. In this regard prepared solutions of copper, nickel and cobalt and laboratory contaminated soils were used to investigate the effects of smelting emissions on the germination and radicle elongation of two deciduous and five coniferous tree species native to north east America. Mineral soils containing in excess of 250 and 500 ppm of each metal prevented radicle elongation in the seeds of the study species. Observations identified that the seeds split but the radicle was killed before it emerged. Experiments undertaken on filter papers impregnated with the prepared metal solutions showed copper and cobalt concentrations below 5 ppm had no effect on radicle elongation when compared with the control and in some cases the metal treatments increased growth. At metal concentrations above 5 ppm all study metals caused increased inhibition of radicle elongation. At treatments above 10 ppm root hairs failed to develop, radicle growth ceased at 50 ppm and the radicle tips died at 100 ppm in all tree species tested. For all species studied the order of metal toxicity was nickel > copper > cobalt in the filter paper studies while nickel and cobalt were more toxic than copper in mineral and organic soil studies. The sensitivity of the individual tree species starting with the most sensitive was birch > honeysuckle > black spruce > red pine > jack pine > white spruce > white pine. This indicated that some deciduous species were more readily damaged than some coniferous species. Deciduous seeds were smaller in size when compared with coniferous seeds and

therefore the increased survival of large seeds was attributed to the greater food reserves present in the larger seeds or dilution of metal in the seed tissue. In conclusion heavy metal pollution can cause a reduction in tree seedling survival and establishment in heavy metal contaminated soils (Patterson & Olson, 1983).

Holub & Zeleñáková (1986) studied the in-vitro influence of Pb^{2+} and Cd^{2+} on pollen germination and pollen tube elongation and reported that low concentrations of the metals caused a slight stimulation of pollen vitality, whereas higher concentrations had an inhibiting influence. The pollen germination and length of pollen tubes was directly influenced by the increasing concentration of metal in the nutrient medium. Pollen germination and pollen tube growth were affected least in woody plants collected from regions of lead pollution. This effect was observed in *Pinus silvestris* growing in soil containing 5000 mg kg^{-1} of lead with tissue lead concentrations of between 1080 and 2260 mg kg^{-1}. This may indicate the gradual adaptation of the reproduction process in response and the evolutionary ability of tolerant populations to increasing lead pollution.

In summary smelting operations emit significant quantities of particulate material which contains heavy metals. These emissions have been responsible for contaminating both soils and vegetation and causing the death of natural vegetation. Previous research at smelter sites has been concerned with the detrimental effects of these emissions on the local vegetation. Studies have been undertaken to determine total soil metal concentrations, metal concentrations in vegetation and the impact of emissions on fertilization and reproduction processes including pollen germination and germination and radicle elongation of seeds. The maximum documented concentrations of metals in soils recovered from such sites were 4000 mg kg^{-1} zinc, 12000 mg kg^{-1} nickel, 9700 mg kg^{-1} copper and 5000 mg kg^{-1} lead. Analysis of foliage from smelter sites recorded the maximum concentrations of heavy metals in the foliage of trees; 2260 mg kg^{-1} lead, 1000 mg kg^{-1} zinc, 506 mg kg^{-1} copper, and <5 mg kg^{-1} cadmium, chromium and nickel. It should be noted these metal biomass concentrations are likely to be influenced more by the fallout and deposition of

material on the surface of the foliage. Uptake and accumulation of metal from soil is likely to be small in comparison.

1.3.3 Mine Tailings

The waste material remaining following the mining or removal of the target mineral is generally deposited in large stock piles termed mine tailings. These materials generally have a poor soil structure, are physically unstable, contain no organic matter and often contain elevated concentrations of toxic metals (Moffat and MacNeill, 1994). Natural colonisation of mine tailings by vegetation is generally recognised as a slow process and is favoured by metal tolerant species (Smith and Bradshaw, 1979).

The waste arising from coal extraction generally comprises a mixture of unburned shale and mudstone, burnt shale and mudstone and wash waste. This type of waste is less toxic and contains fewer chemical contaminants than mine tailings resulting from mineral extraction. The pH of the spoil and source of colonising seeds are important factors which determine the natural vegetation which colonises pit heaps. Early work studying the afforestation of coal mining waste concluded that the pit heap environment was not considered adverse and the major hindrance to colonisation was erosion, surface instability, degree of compaction and acidity in pyritic waste (Hall, 1957).

The successful establishment of both trees and herbaceous species on mine waste was achieved by the neutralisation of acidity, application of required nutrients and deep tipping. However, the growth and survival of trees was poor when compared with grasses and legumes (Shetron & Duffek, 1970).

Metalliferous mine waste unlike coal mining spoil contains elevated concentrations of heavy metals. A survey of metalliferous mine wastes in Great Britain found high concentrations of toxic metals were the major cause of sparse vegetation. The

naturally occurring tolerant population grew faster and persisted longer than introduced plants. The most successful population at each site was those species with the highest tolerance to the toxic metals present at each site. The study identified three cultivators tolerant to various conditions. *Agrostis tenuis* (now *capillaris*) cv *Goginan* was tolerant to acidic lead/zinc waste, *Festuca rubra* was tolerant to calcareous lead/zinc wastes and *Agrostis capillaris* cv 'Parys Mountain" was tolerant to copper waste (Smith & Bradshaw, 1979).

In some studies of trees growing on mine wastes, seed was collected from three tolerant and four non-tolerant genotypes of birch (*Betula spp.*) growing on contaminated mine tailings. The seedlings were studied for extension growth and zinc uptake over a range of zinc concentrations. The extension growth of stems and roots reduced as the concentration of zinc increased. The studies also indicated that in less tolerant genotypes, zinc uptake was limited until a threshold external concentration was reached, above this threshold level the genotype's control mechanism failed to regulate the uptake of zinc and increasing concentrations of zinc entered the roots and shoots. In the tolerant genotypes the regulation mechanism could tolerate higher external concentrations of zinc. Denny & Wilkins (1987b) concluded the experiment identified evolutionary tolerance by *Betula spp.*

Eltrop et al. (1991) studied the lead tolerance of *Betula* and *Salix* species in a mining area in the Mechernich area of the Eifel Mountains. *Salix caprea* L. (goat willow) grew on soils containing up to 17000 mg kg^{-1} total lead and 4000 mg kg^{-1} exchangeable lead while *Betula pendula* Roth (silver birch) was found growing on soils with a total lead content of 29,000 mg kg^{-1} and exchangeable levels of 7000 mg kg^{-1}. Analysis of lead in tree roots revealed a 2.5 fold increase in *Betula* roots (20969 mg Pb kg^{-1} dry weight) compared to *Salix* roots which contained 8467 mg Pb kg^{-1} dry weight. Lead tolerant species not only accumulated more lead in the root but also transferred more lead to the shoots. Lead tolerance in *Betula* was believed to be influenced by plant phosphate status, at low soil phosphate levels the trees accumulated high concentrations of lead in the roots and vise versa. It was also suggested that lead tolerance involves the immobilization of lead by polyphosphate

complexing either in the cell wall or within cells. A calcium dependent mechanism of lead tolerance was suggested for *Salix*.

These studies indicate that some tolerant species of birch and willow are capable of survival in soil with total lead concentrations of 29,000 mg kg^{-1} and exchangeable lead concentrations of 7000 mg kg^{-1} and that heavy metal tolerance exists within some tree species.

1.3.4 Soil Acidity

The widespread dieback of forests in Western Europe within the last 10 to 20 years is thought to be due to increasing emissions of acid gases, including oxides of sulphur and nitrogen generated from the combustion of fossil fuels. Sulphur dioxide and nitrogen oxides dissolve in atmospheric water to form weak acids which are deposited on receiving soil in rainfall. The process is termed acid rain and is responsible for the increased acidification of forest soils and has been highlighted as one of the factors causing forest die back and impaired tree vitality in North America and Western Europe. Soil acidity arising from gaseous emissions from smelters has already been highlighted in Section 1.3.3. The increasing soil acidity also increases the mobility and availability of metals in some soils. The following research has been conducted in an attempt to study the changes in the mobility and availability of metals in acidic soils.

The impact of acid rain and increasing soil acidity on the mobility and availability of soil metals has been widely studied and the relevant findings identified from the literature review are discussed below.

Studies undertaken to determine the availability of aluminium, due to increasing soil acidity, to five different forest tree species found that all five species studied were aluminium tolerant and accumulated high concentrations of aluminium in their roots. Generally the roots of evergreens were found to accumulate more aluminium when compared with the roots of deciduous trees. The results concluded that soil acidification, especially in sandy forest soils caused the high accumulation of aluminium in tree roots. The effect was more pronounced in soils with a low buffering capacity which received high rates of atmospheric deposition of sulphur dioxide, nitrogen oxides and ammonia (Keltjens & Van Loenen, 1989).

In another study components of sugar maple and yellow birch were collected from regions which had been subject to soil acidification from acid rainfall. The collected biomass was analysed for manganese, iron, zinc, copper, lead, nickel and cadmium.

The accumulation of all metals was found to be highest in the foliage and stem bark and lowest in the stem wood, however, because of its large mass, stem-wood was reported to contain the highest concentrations of metal per tree per hectare. Accumulation of metals in order of highest accumulation to lowest were Mn > Fe, Zn > Cu > Pb > Ni > Cd (Hogan & Morrison, 1988).

Soils in Finland are often podzolised, that is to say the upper layers of the soil are more acidic and mobile metals are leached downwards. Podzolisation is a naturally occurring process which can be accelerated by acid rain deposition. The main sources of acidity is either acidic forest litter which accumulates on the floor of conifer plantations or acid rain. As the soil pH increases with depth the mobile metals form hydroxides and are precipitated. When podzolised soils are tilled prior to planting the precipitated metals are brought to the surface and in the acidic conditions they become more soluble and available. Studies concluded that tilling of podzolised soils increased the concentration of aluminium, iron and zinc in birch leaves (Castren et al., 1990).

The soil solution and needles of three native spruce stands in Austria were analysed to investigate the uptake and potential toxic effects of Mn and Al as a result of increased soil acidity. Manganese levels in needles correlated well with Mn levels in the soil while Al levels were similar in all needles analysed. Needle analysis was identified as a good method for estimating available manganese in the soil (Kazda & Zvacek, 1989).

Scherbatskoy et al. (1987) studied the germination responses of forest tree seed to acidity and metal ions. The metals studied included aluminium at 10 and 100 mg/l, cadmium at 1 mg/l, copper at 5 and 10 mg/l and zinc at 5 and 10 mg/l. The experiment concluded that tree seed germination was not reduced by acidity or metals. Seedling germination was generally less affected than seedling development because of the effect of seed food reserves as discussed by Patterson & Olson (1983).

Cox (1988a) studied the effects of acidity and trace element components of polluted rain with regard to pollen germination and pollen tube growth (in vitro). The study found that acidity caused a significant inhibition of germination and germ tube growth on studied pollen, especially in combination with copper. The study of separate metals found that lead significantly inhibited both germination and growth of the studied pollen whereas zinc had little or no effect. In conclusion increasing soil acidity may increase pollen mortality and could have significant implications for plant reproduction and breeding. Pollen germination was further studied in regard to wet deposition of acidity and copper on various fruit and seed set parameters. A significant negative correlation was found between pollen germination, acidity and copper, however, the response in the absence of copper showed no clear correlation. The impact of such pollution may be increased fruit abortion and reduced number of seeds per plant.

In conclusion soil acidification has been shown to increase the mobility and availability of metal ions within the soil environment. This increasing acidity results in the increased uptake of mobile metals by vegetation growing in acidified soil. This indicates the uptake and accumulation of heavy metals by vegetation may be more pronounced in acidic soils and the importance of soil acidity should be recognised as an important factor which is likely to influence the phytoremediation process.

1.3.5 Nutrient Studies

The growth and health of vegetation is dependent on the availability of both micro and macro nutrients in the correct quantities (Wild, 1988). In forest systems the collection and analysis of foliage samples is an adopted procedure for determining the nutrient status of individual trees and determining the fertilizer requirement of the forest. In this regard studies have been conducted to determine the seasonal variation of elements (nutrients and heavy metals) in the foliage of trees. In forest systems nutrients which remain in the leaf prior to or at leaf fall are returned to the

soil and leads to a cycling of nutrients in the forest environment. Studies have also been undertaken to determine the concentration of nutrients returned to the soil from leaf fall.

The factors which influence the mineral content of leaves are varied and include; the nature and status of the soil, plant species and genotype, the age of the plant and the position of the leaf on a tree (Moffat & McNeill, 1994). The variation and concentration of an element with time at a given site is much less than the variation found between different sites. In this regard site properties are believed to have more influence on the metal contents of tree biomass.

The seasonal variations of metals in 0 to 4.5 years old twig samples was studied by collecting samples from shaded and unshaded positions on experimental trees (Fromm et al., 1987). Macro nutrients were generally found to increase in concentration during the growing season while trace elements increased in concentration and reached a maximum concentration in July. Calcium, magnesium, manganese and zinc were the most abundant metals in birch leaves. Copper and nickel were found to decrease with increasing growth but this was attributed to a dilution effect due to dry weight increase. The concentrations of manganese, iron, zinc and lead were analysed in the tissue of beech leaves collected during autumn. The concentration of study elements was found to decrease in the senescing leaves and was attributed to either the retrieval and re-adsorption of the elements by the tree or leaching from the leaf surface by rain or dew. The major reservoirs of heavy metals in the stem were identified as the periderm, cortex, pith and xylem rays. In long lived trees the concentrations of manganese, iron, zinc and lead were recorded to increase in the veins of leaves during the period when they changed from green to brown autumn colouration. This process may signify the elimination of heavy metals from the plant body The concentration of manganese, iron, zinc and lead also accumulated in the sieve element/companion cell complexes of the leaf veins during the period of autumn colour change and may indicate the self purification mechanisms of trees involving the exclusion of toxic materials by deposition into bark (rhytidom), the cortex of roots, drying branches, fruits or autumn leaves.

In a similar study first and second year Norway spruce needles were collected over a period of nine separate dates throughout the year at four different sites. The collected samples were analysed for twenty different elements. Following analysis the 20 elements were categorized into three separate groups on the basis of their seasonal fluctuating concentrations as follows:

- Group 1 calcium, strontium, barium and manganese
- Group 2 aluminium, bromine, cobalt, iron, mercury, lanthanum, scandium, lead and zinc
- Group 3 potassium, rubidium, cesium, phosphorus and chlorine

Magnesium and sodium did not fall within any of the groups. The concentration of elements in both group 1 and 2 increased with time throughout the growing season. Group 2 elements generally increased with a linear response with respect to time in both young and old needles. No increasing of decreasing trend with respect to time was evident in those elements in group 3 (Wyttenbach & Tobler, 1988).

The concentration of essential elements (Ca, Cl, Cu, Fe, K, Mg, N, P & Zn) and non-essential elements (Al, As, Ba, Br, Co, Cr, Cs, Hg, I, La, Na, Rb, Sb, Sc, Si and Sr) were determined in five successive needle age classes of Norway spruce (Wyttenbach et al., 1995). A total of 40 mature spruce trees from six different sites were investigated individually. Trees from similar sites had similar dynamic behaviour and it was possible to predict the behaviour of different groups of metal at different sites with the exception of Mn, Co, Zn, Ca, Sr and Ba.

Studies by Young (1971) on the bark percentages and chemical elements in complete trees reported a 4.6 : 1 ratio of metals in bark to wood. Almost every chemical element was present at higher concentrations in the bark than the wood. It was concluded that by removing both wood and bark from economic forests removed approximately 50% more nutrient than just removing the wood.

The retention time of N and P was studied in leaf biomass. The retention time of both nutrients appeared to be related to nutrient status, longer retention was noted during periods of low nutrient availability. Increase in leaf longevity was also recognised as the best adaptation for increasing efficient use of nutrients (Escudero et al., 1992).

The levels of nutrients and trace elements were determined in the foliage of nine tree species grown on a mixture of pulverised fuel ash (PFA) and gypsum. The studies found no serious micronutrient deficiencies in the study plants. PFA was found to increase foliar levels of boron, potassium and molybdenum (Shaw & Moffat, 1993).

The previous studies have shown that the collection and analysis of forest foliage is a widely adopted procedure for determining the nutrient requirements of commercial woodlands. The findings of such studies have shown the seasonal accumulation of mercury, lead and zinc in the foliage of trees. This accumulation of metal in the foliage of trees indicates the removal of metal from the soil and transfer into tree biomass, however, it should also be noted heavy metals which accumulate in leaf tissue prior to leaf fall will be returned to the soil at leaf fall. Where this recycling of metals is considered a problem it may be worthwhile considering the removal of leaf material along with timber to prevent the accumulation of metals in the surface layers of the soil. which may make the issue of soil contamination worse by concentrating heavy metals within the surface layers of the soil in a more available form. These studies again indicate the uptake of metals by plants and the potential of phytoremediation.

1.3.6 Human Health Implications

In the assessment of the risk of any environmental contaminant the first hazard considered is often human health. Heavy metals in the environment can come into contact with humans by a number of pathways including direct contact, inhalation and ingestion. The greatest risk to human health resulting from heavy metal uptake

by vegetation will usually be through the harvesting and ingestion of contaminated vegetation or soil, especially by young children (ICRCL, 1989). Further risk of heavy metal ingestion can occur when heavy metals are accumulated in edible fruits or seeds and those fruits and seeds enter the human food chain.

The ingestion of heavy metal contaminated fruits by humans represents direct uptake and during the redevelopment of contaminated land it is crucial that the reclamation techniques adopted are effective in reducing or eliminating such pathways.

One widely adopted procedure adopted to reduce the risk of metal uptake by vegetation is the provision of a capping layer of uncontaminated soil over the contaminated soil. The importance of the thickness and composition of contaminated land capping materials in reducing the uptake of heavy metals by fruit crops has been studied by Harris et al. (1983). The heavy metal content of the edible and foliar components of fruit crops were analysed with respect to 10 different capping treatments. The results indicated that of the species studied, raspberries were found to contain elevated concentrations of metals, especially cadmium, in both the fruit and foliage to levels which could be considered a risk to human health. The accumulation of heavy metals was found to be worst in perennial crops which tended to have extensive root systems which could penetrate some of the capping layers. The assessment of the effectiveness of the capping layer was found to be related to cap thickness and its ability to restrict root penetration.

Further to concerns that the edible parts of plants may contain heavy metals at hazardous concentrations, the adsorption and accumulation of cadmium, nickel and chromium in different components of tomato (*Lycopersican esclentum mill*) was studied in nutrient solutions (Moral et al., 1994). Metal treatments were prepared from chloride salts at the following concentrations in mg l^{-1}, Cd^{2+} (0, 10 and 30), Ni^{2+} (0, 5, 15 and 30) and Cr^{3+} (0, 50 and 100). The highest concentrations of metals accumulated in different components of tomato plants and within fruit were as follows (results expressed as mg kg^{-1}).

	Roots	Stem/branch	Leaves	Fruit
cadmium	973	157	141	4.8
nickel	1016	406	59	49
chromium	2354	14	59	not detected

Chromium was poorly transported to fruits while cadmium and nickel were more easily transported and accumulated to higher concentrations in stem, branches, leaves and fruit. The experiment concluded that elevated concentrations of cadmium and nickel in soil could be a serious risk to human health.

Heavy metal uptake in vegetation does not only pose a risk to humans but to other organisms. The content of heavy metals in both the leaves of plants and aphids feeding on those leaves was analysed and no significant accumulation of metal was recorded in aphids although differences were found in metal contents between leaves, attacked leaves and aphids (Spicarova, 1985).

These studies demonstrate the potential for vegetation to accumulate trace and heavy metals in above ground foliage and biomass and the risks that this accumulation can cause to animals including humans. Again this accumulation of heavy metals within plants highlights the phytoremediation of vegetation.

1.3.7 Environmental Dating

The ring wood of trees has been identified as discrete or identifiable layers which have accumulated after each growing season (Rolfe, 1974). This discrete deposition of information with time is similar to the deposition of polar ice, snow layers in glaciers, sediments and peat bog deposits. In all these cases a sample taken from a discrete layer can be dated and the analysis of the sample can often reveal environmental conditions that existed at the time of deposition.

Growth rings in trees arise from differences in the rates of cambium divisions during various seasons of the year (Weier et al., 1982). Nutrients including non-reducing sugars are translocated in the phloem while water and solutes are transported in xylem. The death of many cells in secondary tissues has been attributed to the accumulation of waste metabolites. The most effective means of excreting waste is via ray cells which increases heavy metal concentrations in non functioning tissue of heartwood and outer bark. Although ring wood is generally laid down on an annual basis, it is generally accepted the layers are not discrete as the tissue is alive and there may be some transfer of metals between different layers. Ray cells are thought to be an excretion route for toxic products and heavy metals are thought to accumulate in the xylem. In addition it is often difficult to date growth rings as some may be missing or more than one growth ring may form in a year (Stewart, 1966).

The elemental concentrations in tree rings have been used as a means to monitor metal contamination, fertilization and the effects of acid rain in time. When selecting suitable study trees for dendrochemical research it is important to select species which minimise radial translocation of heavy metals. The selected species of tree should also be long lived, grow on a wide range of sites, have distinct heartwood, a low number of rings in sapwood, low heartwood moisture content and low radial permeability (Cutter & Guyette, 1993).

Okada *et al* (1993a and b) studied the radial distribution of trace elements in 20 tree stems and found that elemental contents were larger in sapwood than heartwood in hardwood species while contents in softwood stems were larger in heartwood when compared with sapwood. Chemical analysis of the stems of seven softwood species found the concentrations of alkali metals were higher in heartwood than sapwood. Manganese and chloride were higher in sapwood.

The potential of tree ring as indicators of environmental lead accumulation was used to study the release of lead from auto mobile exhausts. In the study sugar maple (*Acer saccharum Mangh*), red oak (*Quercus rubra L.*) and loblolly pine (*Pinus taeda L.*) stem cores were collected from trees growing near and remote to automobile traffic. The experiment found trees growing adjacent to roads contained significantly higher concentrations of lead than trees located remote from traffic (Rolfe, 1974).

Lead, cadmium, zinc, copper and manganese levels were analysed in the ring wood of trees. The study identified a significant increase in the concentration of lead, zinc, copper and cadmium in tree ring wood around 1860-1870 in trees growing in urban environments. This period coincided with the beginning of urbanisation and industrialisation. A second increase in metal contents was recorded around 1950 and was attributed to the increasing use of the motor car and leaded petrol. The process of metal accumulation was attributed to either the deposition of metals on the soil and uptake through the root system and xylem or the direct deposition onto the external surfaces of the tree followed by transport into the ring wood. Given that lead would be relatively immobile in the soil the most likely route would be incorporation of air borne metal trapped by the bark (Stewart et al., 1991).

In a similar experiment tree rings and bark collected from trees growing at various distances from highways were analysed for lead, copper and zinc. The study found the concentration of lead, copper and zinc decreased in trees progressively located at greater distances from main roads. Metal contents were also recorded to decrease with increasing height above ground. These observations were attributed to general traffic density at the sites where the levels of lead in tree bark were recorded at concentrations between 100 and 700 ppm (Barnes et al., 1976).

The radial distribution of cadmium, copper, lead and zinc was studied in the annual growth rings of oak growing within two regions; an unpolluted region (Valadivia, Chile) and a polluted area (Konigstein). The studies found no significant difference between the concentrations of copper and zinc between the two sites, however

cadmium and lead levels increased by a factor of 2 and 12 respectively in trees growing in the polluted area. The greatest increase in metal concentrations was observed in the outer growth rings bordering the cortex but there was no significant difference recorded between metal concentrations in heartwood and sapwood. These findings have been explained as either the radial displacement of metal or metal exchange between the cortex and the most external growth rings (Querolo et al., 1990).

Meisch et al. (1986) analysed the annual growth rings of 140 to 160 year old beech trees for a total of 14 elements by atomic absorption spectroscopy. Based on the results the elements were segregated in to three groups. Group 1 contained metals which had no tendency for chronological change (Na, K, Cu, Cr, Co, Ni and Pb). Group 2 contained metals which decreased in concentration (Ca, Mn and Zn), and Group 3 contained metals which had a tendency to rise in concentration (Fe and Al).

Although in real terms the levels of metal held within stem-wood are relatively low, the environmental dating studies indicate the long term retention of metal in the wood of trees. This may be an important consideration when using woodland species to remediate heavy metal contaminated soil as metals held in wood will be removed within harvested timber.

1.3.8 Metal Movement in Vegetation

Studies which segregate and analyse different components of vegetation are able to record the concentration of metal in that component at a given time, however component analysis studies do not determine the route by which metals are transported throughout the plant.

Weier et al. (1982) suggests plants have no specific uptake mechanism for water. Water uptake is in response to physical forces and therefore movement is from regions of high water potential to regions of low water potential. During this bulk

transport of water, metal and non-metal ions are likely to be transported in the water. The uptake of water by plant roots occurs primarily in the region behind the root tip where root hairs develop.

In situations where there is no bulk transport, passive and active transport mechanisms are required to transport ions into plant cells. Passive transport is primarily by a process of diffusion while active transport requires carrier proteins on cell membranes. The location of ion uptake in roots is not defined and some metals are taken up only at the apical region while other metals are taken up over the entire root surface. Metal uptake is also dependent on the uptake capacity and growth characteristics of the root system. Plants can also prevent the influx of certain metals, and it has been noted that plants accustomed to acid soils possess mechanisms to prevent the influx of Al^{3+} ions into the root by forming organic-aluminium complexes or binding/precipitating Al^{3+} ions in the cell wall of the root cortex. Once ions have been transported across the external cell membranes of the root the ions can be transported within the plant. Ion transport within the root is documented to occur by two different methods (Weier et al., 1982);

- apoplast pathway - movement is exclusively through cell walls avoiding passage across membranes.
- cellular pathway - movement is from cell to cell through the membrane.

The uptake of metal ions by epidermal and cortex cells is considered to be by active transport, however, the transport mechanism into xylem is unknown.

Once in the xylem the velocity of water and solute through the xylem occurs at approximately 1 mm/s in trees during periods of transpiration. As a result water and ions taken up by the root are transported through the plant in the xylem to above ground components. Xylem comprises dead cells and allows the free movement of solvent and solute. The driving force behind the movement of water is the diffusion of water through the leaf and evaporation at the stomata, termed transpiration. It

should be noted that when the water evaporates from the leaf the solutes that were carried in solution are left behind in the leaf.

Phloem is living tissue that translocates the products of photosynthesis from the point of manufacture in leaves to areas of growth and storage. It also important in the redistribution of plant growth regulators and other compounds.

Heavy metals complexed with organic ligands such as citrate can usually be transported in the xylem. Other plant nutrients such as sodium, potassium and magnesium are easily transported in the phloem whereas iron, manganese, zinc and copper are only moderately mobile.

Although the uptake of heavy metals is poorly understood, copper and zinc have been reported to enter plant cells by metabolic pathways while non-essential metals like cadmium and lead are more likely to enter cells by a combination of both metabolic or non-metabolic mechanisms (Dixon, 1988).

Studies carried out to determine the enrichment of chemical elements in plant roots found that the uptake of major nutrients, like potassium, required active transport. Active transport was defined as osmotic work done at the expense of metabolic energy. The study also found that zinc uptake by roots from nutrient solutions increased after the roots had been killed. This indicates that accumulation of zinc by plant roots can be a passive process (Findenegg & Broda, 1965).

It has been reported that copper, nickel and zinc are never present in a free ionic form in xylem sap but are generally present as some anionic complex with either an organic acid or amino acid. In herbaceous plants lead was found to be bound to the xylem but as the concentration of unbound lead increased in the xylem vessels, more lead accumulated in the leaves. Lepp & Dollard (1974a) used radio isotope tracing procedures to study the movement of metals within plants. The lateral movement of lead was studied within woody stems using the radio isotope ^{210}Pb. The findings of the study identified the lateral movement of ^{210}Pb from bark to wood in all species

studied with the lead binding to the cell walls of xylem vessels. Further studies on the binding of lead to xylem tissue showed that free lead ions bind more readily to xylem than organically complexed lead (lead-glycerin). The lead was also observed moving laterally from wood to bark.

In another study the uptake and distribution of mercury was studied within higher plants using radio-tracer techniques. The study plants tolerated an external solution concentration of 1 mg kg^{-1} of mercury and uptake generally corresponded with the solution concentration. On average 95% of the mercury taken up by the plant was stored in the roots compared with the shoots. Attempts to extract the mercury using ethanol and hydrochloric acid identified two separate pools of metal, a fraction which was removed and one which was not. A procedure of cell fractionation identified the major binding component for mercury was the plant cell wall. In addition the treatment caused significant inhibition to plant growth including reduced shoot and root length and a reduction in water uptake. The conclusion of the study was that plant roots were a significant absorption site for mercury and therefore restricted further transport of mercury through the plant to the foliage (Beauford et al., 1977).

The research work discussed would appear to indicate that free ions are likely to be transported along with water movement within plants. However, due to the binding of heavy metals with organic material including cell walls in the roots, significant free movement of heavy metal cations in not normally observed. When heavy metals become chelated with soluble organic chelates the heavy metal chelate can be soluble. Such organo-metal chelates can be transported in the xylem and phloem. In this regard chelated heavy metals are considered to be more mobile within plants and as a result organic chelates could be important in influencing the accumulation of heavy metals in vegetation.

1.3.9 Metal Resistance Studies

Through the process of natural succession plants have colonized metalliferous mine wastes and other contaminated sites which contain elevated concentrations of heavy metals. This adaptation of plants to these unfavorable environments is thought to be due to the possession of resistance, however the process of metal resistance is poorly understood.

The evolved tolerance of plants to toxic concentrations of heavy metals is a recognised phenomenon in plants inhabiting spoil heaps/mine tailings. The process of initial tolerance termed natural selection is rapid and identifies genetic variance in non-tolerant populations. Such tolerance genes are often considered disadvantages to growth in uncontaminated sites (MacNair, 1987).

Many derelict sites exist in urban/industrial areas. The methods adopted by trees in such environments to allow them to survive in polluted environments are varied and generally require possession of neither too much nor too little tolerance to allow optimal growth. Development of tolerance has been observed in plants where roots proliferate in the fertile zone of soil and avoid the zones of worst contamination. Avoidance strategies can also be genetically determined including variations of external leaf or stomatal surfaces which reduce adsorption of gaseous pollutants. At a molecular level some proteins and metal binding peptides can be induced by exposure to heavy metals (Dickinson et al., 1991).

There have been suggestions of two separate methods of resistance adaptation in plants, termed either metal avoidance or metal tolerance. Tolerance is believed to require possession of specific physiological mechanisms to control the concentration of metal entering the plant and is believed to function through a process of either metal exclusion or restricted metal uptake and transport. Metal accumulation on the other hand generally occurs when there is no restriction on metal uptake and the metals accumulated by the plant are stored in a detoxified form within the plant. Processes of detoxification include cell wall binding, active pumping of ions into

vacuoles, complexing with organic acids and metal binding proteins, enzymic adaptations and membrane permeability (Baker, 1987).

Other adopted methods of detoxification by plants to prevent metal toxicity include; the formation of insoluble crystals and vesicles containing heavy metal, dilution into non-vital organs and accumulation in root cells (Dixon, 1988).

The following observations were reported by Wilkins (1991) following studies undertaken to determine the tolerance of vegetation to heavy metals.

1. Tolerance is largely metal specific
2. In breeding experiments tolerance is frequently found to be dominant over sensitivity
3. Tolerant plants frequently accumulate less metal in shoots but more in roots than non-tolerant relatives
4. Metal toxicity symptoms generally occur due to the nature of the substrate and presence of other ions rather than absolute metal concentrations.
5. Metal tolerance relies on complexing of metal ions and accumulation in a non-toxic form in cell walls or vacuoles.

With reference to item 4 above the uptake of heavy metals has been shown to be influenced by the presence and availability of other metals and plant nutrients. Nitrogen, phosphorus and potassium have been reported to directly influence heavy metal uptake by vegetation. Increasing lead toxicity has been observed in nutrient solution experiments with the removal of phosphate. However it is thought that the phosphate complexes with the lead in solution and precipitates the lead as lead phosphate which reduces the plant uptake of metal.

The acclimatization of trees to pollution stress was studied by collecting vegetation samples from sites contaminated by the aerial deposition of copper and cadmium. Cell cultures were cultivated in nutrient solutions containing elevated concentrations of copper and cadmium (10 and 15 mg l^{-1}). Both metals were found to inhibit the growth of species collected from uncontaminated sites but no growth inhibition was

noted in species collected from contaminated sites. Tolerant species were found to remove less metal from the nutrient media compared with non-tolerant species. The study confirmed that trees growing on contaminated sites can evolve a level of tolerance to the unfavorable conditions (Dickinson et al., 1992).

Sixteen willow (*Salix*) clones were screened for resistance to copper in solution culture using copper concentrations of 0.25, 0.50 and 0.75 mg l^{-1}. Significant differences were reported in root length, number of lateral roots produced and patterns of metal uptake between species, hybrids and clones. *Salix caprea* and *Salix cinerea* and their hybrids with *Salix viminalis* grew best in the elevated copper solutions (Punshon et al., 1995).

Such resistance studies show the ability of plant species to adapt and survive in contaminated environments. The main processes of tolerance is believed to be either metal tolerance or metal avoidance. The process of metal avoidance requires the exclusion of metal from the plant and in this regard tree species which practice metal avoidance will be unsuitable for bioremediation. Tree species which are metal tolerant, especially those that accumulate and store metals within their biomass will be better suited for bioremediation.

1.3.10 Root Mycorrhizae

The importance of mycorrhizal fungi in vegetation nutrition studies has been reported to increase the uptake of water and nutrients including phosphate and nitrogen. On the basis that mycorrhizal fungi enhance the uptake of macronutrients and micronutrients in vegetation it has often been assumed that they will enhance the uptake of harmful non-nutrient metals causing toxicity problems. However, studies of heavy metal uptake has shown that ectomycorrhizal infection of tree roots actually reduces the uptake of certain heavy metals from soil (Wilkins, 1991).

Soils containing above normal levels of heavy metals have also been reported to decrease the incidence of mycorrhizal infection of root tips which has the effect of decreasing root distribution and biomass accumulation. The impact of heavy metals on the incidence of root mycorrhizae is also reported to be more severe than soil acidity (Bell et al., 1988).

Investigations have shown that mycorrhizal plants take up less calcium and potassium and consistently accumulate less cadmium, nickel, lead and zinc than non-mycorrhizal counterparts. However no improvement in growth was noted between mycorrhizal and non mycorrhizal plants. When analysed the cells of mycorrhizal roots contained significantly less zinc in both the cortex and stele while increased concentrations of zinc was located in the extramatrical mycelium. These observations were reported to show the interception and binding of zinc by root mycorrhizae. Such binding is thought to decrease the toxicity of particular metals to host trees by intercepting and reducing the concentration of metals (Zn, Ni, Al, Cu and Ca) in root and shoot tissue (Wilkins, 1991).

The importance of mycorrhizal fungi for the establishment and development of trees on mine tailings has been raised. Seedlings with abundant ectomycorrhizae have been observed growing rapidly in toxic mine overburden and has been attributed to the protection of the roots by the fungal mantle. Studies of willow and poplar growing on mine tailings found non-mycorrhizal trees were generally shorter in height, showed little growth, and possessed root systems that were shallow and rootlets that were long and thin. These findings were attributed to the trees being unable to obtain the required quantity of nutrients, although the possibility of metal toxicity was appreciated as an influencing factor (Harris & Jurgensen, 1977).

Soil cadmium and nickel levels in excess of 200 mg kg^{-1} and lead levels in excess of 50 mg kg^{-1} have been shown to prevent ectomycorrhizal formation. The addition of cadmium, nickel or lead at concentrations of 10 or 20 mg kg^{-1} was reported to reduce the dry weight and leaf area of non-inoculated plants more than inoculated plants. The total dry weight and leaf area of ectomycorrhizal *Quercus rubra* seedlings was

53 and 35% greater when compared with non-inoculated seedlings. At metal concentrations in excess of 50 mg kg^{-1} significant growth reductions were observed in inoculated plants. Inoculation also increased the content of phosphate, magnesium, zinc and iron in roots and leaves. The concentration of cadmium, nickel and lead increased in leaves and roots of non-inoculated plants (Dixon, 1988).

The ectomycorrhizal amelioration of zinc toxicity was reported in ectomycorrhizal *Betula* spp. The infected trees had lower concentrations of zinc in their root tissue than their non-mycorrhizal counterparts. The zinc was found to be associated with the extramatrical mycelium, bound to either the cell wall or extrahyphal slime polymers. This removal of zinc by root mycorrhizae lowers the zinc concentration in the soil solution surrounding the root which in turn decreases the uptake of zinc in the roots (Denny & Wilkins, 1987a).

Ectomycorrhizal fungi are one of many types of soil microorganisms which can be influenced by heavy metals in soil. Increasing concentrations of heavy metal have been shown to prevent the establishment of ectomycorrhizal fungi. It is therefore likely that heavy metals in soils will have a toxic effect on other soil living microorganisms. The accumulation of Pb, Zn, Cd and Cu in forest litter close to a lead-zinc-cadmium smelter was attributed to emissions from the smelter. Studies found that these toxic metals were causing a reduction in the number of soil microorganisms which in turn was reducing organic matter decomposition. The study concluded that smelter emissions were causing a build up of organic matter in affected soils (Coughtrey et al., 1979).

Nordgren et al. (1986) measured the microbial activity of soil to determine the influence of increasing heavy metal concentrations. The bacterial numbers in soil collected near to a smelter were reduced 8 to 11 fold when compared with typical populations in uncontaminated soil. Chitin hydrolysers were 5 times less abundant, soil respiration and urease activity was decreased by a factor of 4 and soil respiration rates fell from 100-150 to 30 $\mu g\ CO_2$ (g dry soil)$^{-1}$ h^{-1}. Phosphatase activity and mycelial lengths were unaffected in contaminated soil. The increasing concentrations

of soil heavy metals and decreasing soil pH were the two main factors attributed to the findings.

Tyler (1974) studied the activity of hydrolytic soil enzymes in spruce mor which had been polluted with zinc and copper emissions from a brass foundry. Increasing concentrations of copper and zinc were found to decrease enzyme activity, respiration rate, urease and acid phosphatase activity. The reduced soil enzymic activity was causing a build up of organic matter in heavy metal polluted forests.

Williams et al. (1977) investigated the decomposition of metal tolerant vegetation growing on mine waste and compared the findings with the decomposition of similar vegetation on an adjacent uncontaminated site. The high concentrations of lead and zinc which accumulated in grass growing on mine waste was reported to retard decomposition. The effect was shown by increased accumulation of litter, reduced humus formation, reduced soil urease activity and smaller microbial and microfaunal populations on contaminated sites.

Giller et al. (1993) created a gradient of increasing soil heavy metal contamination by mixing farmyard manure and contaminated sewage sludge and inoculating the prepared soils with strains of *Rhizobium.* The study found that relatively small concentrations of heavy metal were effective in killing all free-living *Rhizobium,* however, after formation of root nodules the rhizobia appeared to be protected and were more tolerant to increasing heavy metal concentrations in the soil. This reduced vitality of rhizobium in heavy metal contaminated soil was attributed to a 40% reduction in dry matter yield of white clover on heavy metal contaminated soil.

Root mycorrhizal studies indicate that while such associations can be important in increasing macro and micro nutrient availability to plants they do not increase the availability of toxic heavy metals. The studies undertaken suggest that the presence of root mycorrhizae actually reduces the uptake of heavy metals by infected plants. The reduction in heavy metal uptake is thought to be due to the binding and immobilisation of metal in the external surfaces of the extramatrical mycelium. In

this regard root mycorrhizal associations are likely to reduce metal uptake in bioremediation studies. However, root mycorrhizal associations have also been shown to reduce the toxic effects of heavy metals to vegetation. In this regard root mycorrihizal associations may be beneficial for establishing vegetation on difficult and contaminated sites.

1.3.11 Establishment of Vegetation on Derelict Sites

In the United Kingdom many industrial activities which included coal mining, quarrying activities, the manufacture of chemicals and iron and steel works produced waste materials which were often tipped into spoil heaps as a means of disposal. The spoil tips arising from mining and quarrying activities often comprised mixtures of the following materials:

- bind - an industrial clay mixed with oxide of iron
- fireclay
- sandstone
- ironstone
- clay and boiler ash

Early studies by Brierley (1955) on the establishment of vegetation on coal bings found that vegetation was generally found in areas where the surface material was most stable. Fine granular material was believed to offer no shelter from wind and water erosion and became dense at the surface when wet causing penetration problems for roots and shoots. Stable anchorage positions for vegetation appeared to be provided by the presence of large rocks. The findings suggested the establishment of vegetation on coal bings was dependent on mechanical rather than chemical factors. In addition the total number of plants per unit area increased with the age of the bing.

During early reclamation projects sites were generally reclaimed for amenity purposes including landscape creation and provision at parks and recreation grounds.

This remediation of derelict sites was often achieved by planting trees either for economic timber production or amenity parkland. Weathered spoil mounds particularly those comprising easily decomposed materials including some shales and burnt fire clay were generally easier to vegetate than other sites with poorer soils. The selected vegetation for remediation works was required to have the characteristics of pioneer species including low nutrient requirements, vigorous and penetrating root growth and the ability to increase the nutrient and organic matter content of the soil. Common reclamation species included; gorse and broom which were efficient nitrogen fixers and Corsican pine which survived in dry and smoky conditions (Sisam & Whyte, 1944).

Casson & King (1960) reported that the afforestation of derelict land arising from mineral workings in Lancashire has produced commercially viable timber crops when the correct species of tree were selected. Standard forestry management practices were found to be suitable on such sites. The main problems to establishment were the physical surface instability of colliery waste tips and pH and toxicity problems in wastes containing pyrite and sulphates.

Most of the early reclamation works on derelict sites concentrated on the establishment to woodland. These woodlands were created for both amenity and commercial purposes. The ground conditions encountered were generally unfavorable, however, the sites were not considered to be contaminated by heavy metals. These early reclamation works are not directly relevant to the selection of vegetation for bioremediation purposes, however, they do identify a number of physical constraints which are likely to be present on many contaminated sites.

1.3.12 Metal Uptake by Vegetation

The section summarises the findings of metal uptake/response studies by/with trees and other vegetation reported by various researchers. The relevant studies will be discussed in the order of field studies and laboratory studies.

Field Studies

Plant regulation of essential and non-essential metals is an important control mechanism required by vegetation growing on soil which contains elevated concentrations of metals. This regulation or more commonly non-regulation has been used beneficially for biogeochemical prospecting and assessing pollution of mine wastes and smelter emissions. Leavitt et al. (1979) studied the regulation of two essential elements (Cu and Zn) and three non-essential elements (Ag, Cd and Pb) in different components of trees growing in contaminated soil and reported:

- copper, zinc, cadmium and lead were recorded at lowest concentrations in foliage
- the concentration of cadmium and lead in twigs increased with twig age
- species which contained high concentrations of zinc also contained high concentrations of cadmium and conversely species which contained low zinc concentrations also contained low concentrations of cadmium.
- The concentration of silver, copper and lead in plant ash was higher than in soil. The concentration of silver, copper and lead in dried plant biomass was less than the metal concentration in the soil, however the zinc dry weight content of vegetation was generally greater than the concentration in the soil.

Field trial studies undertaken to determine the concentration of metals within trees and vegetation are likely to provide the best indication of achievable metal uptake levels. The uptake of copper and nickel by birch trees was studied at two different sites and was ascertained by analysis of collected leaf and twig samples. The studies found that plants growing in copper and nickel contaminated soil accumulated higher concentrations of the respective metal and as a result plant analysis could be used as a means of biogeochemical prospecting (Ehlin, 1982 and 1983).

Biomass production, root penetration and heavy metal uptake was studied in birch trees growing on copper tailings. Pyrite oxidation in the mine tailings had acidified

the soil and increased the solubility of the heavy metals. The main factors studied during the investigation were:

- the effect of soil cover thickness over the mine tailings on the upward transport and content of heavy metals in soil
- root distribution of the study trees
- heavy metal content in the study trees
- transport of heavy metals

The mine tailing had been capped with clean soil approximately 10 years previously and since had been colonised by a number of birch and pine trees, the largest of which were approximately 4 metres tall. The maximum metal concentrations recorded in the spoil was 14000 mg kg^{-1} of zinc, 2100 mg kg^{-1} copper, 12400 mg kg^{-1} lead and 48 mg kg^{-1} cadmium. The metal concentrations in the cover soil was much lower. The narrow zone of cover soil in direct contact with the spoil contained elevated concentrations of water soluble heavy metals. Most of the fine root biomass was found to be located in the upper regions of the soil cover and root proliferation decreased with depth, especially when the soil cover was shallow. Where the upward transport of metals from spoil to the capping layer of soil had occurred the zinc, lead and cadmium contents of the study trees were higher than for trees from uncontaminated sites. The uptake of cadmium and lead was correlated to metal concentrations in the soil, while zinc foliage concentrations were influenced by soil pH and the thickness of capping (Borgegard & Rydin, 1989).

The distribution of heavy metals within a woodland ecosystem which had been contaminated by lead/zinc smelting operations was studied by collecting and analysing leaf, bark and twig samples. The concentration of lead, zinc, cadmium and copper within trees situated approximately 3.2 kilometers downwind of the smelter was greater than concentrations in an uncontaminated woodland. Ground flora had higher concentrations of cadmium and zinc while tree leaves contained more copper and lead. From the weight of biomass and recorded metal concentrations it was estimated that the contaminated woodland system contained 1.46 g/m^2 cadmium, 43.3 g/m^2 lead 120.2 g/m^2 zinc and 14.5 g/m^2 copper. In the woodland ecosystem

root biomass was reported to account for approximately 14% of total biomass but hold between 20 and 40 % of biomass lead and zinc. Heavy metal contamination of vegetation was attributed to contamination by atmospheric deposition rather than plant uptake. In the soil heavy metal concentrations were found to decrease down the soil profile and approximately 78% cadmium, 60% lead, 59% zinc and 26% copper were present within the top six centimetres of soil (Martin et al., 1982).

Gemmell & Goodman (1980) undertook large scale field trials to study the growth of trees on zinc smelter waste following soil applications of PFA and organic material in the lower Swansea Valley, Wales between 1965 and 1970. The study found that the growth of plants generally declined in the period 1968 to 1969 and was attributed to zinc toxicity. PFA coverings of 15 and 22.5 centimetres with annual N, P, K fertilizer applications was the most successful treatment for increasing tree growth, especially for zinc tolerant populations.

Luwe (1995) analysed forest floor vegetation growing in Beech (*Fagus sylvatica L.*) stands in Germany. The concentrations of macronutrients (Ca, Mg and K), micronutrients (Fe, Mn, Zn and Cu) and phytotoxic metals (Pb, Cd, Ni and Al) were measured in soil, roots, rhizome, stems and leaves of forest floor species (*Mercurialis perennis L.* and *Polygonatum multiflorum L.*). Ammonium chloride extractable cations, pH and other soil variables were also measured. The highest concentration of micronutrients were found in leaves, while phytotoxic metals accumulated in roots. The amount of heavy metals extracted with ammonium chloride increased with decreasing soil pH. The author concluded that elemental uptake by the study plants was indirectly controlled by the pH of the upper mineral soil.

Gustafsson & Eriksson (1995) studied forest soil chemistry and tree bark chemistry in forest systems growing near Uppsala, Sweden. The studies found a positive correlation between bark chemistry (total Ca, exchangeable Ca and total Mg) and soil chemistry (pH, soil exchangeable Ca, soil exchangeable Mg). The author

concluded that soil had an important bearing on the transportation of nutrients from tree roots to bark.

Laboratory Studies

The following text is a short summary of various studies undertaken by various researchers studying the uptake of various metals by different study vegetation. The growth of beans, carrots tomatoes and grasses were studied in soils which were contaminated by varying concentrations of a wood preservative containing copper, chromium and arsenic. Soils with an additive Cu/ As/ Cr concentration of 7000 mg kg^{-1} completely inhibited the growth of all species studied. Carrots grown in soils containing 1000 ppm Cu and Cr and 200 ppm As produced crops containing twice the recommended limit for As in food. In field studies *Equisetum arvense* (non woody) was observed growing adjacent to preservative contaminated soil containing 7000 mg kg^{-1} Cu, 6900 mg kg^{-1} As and 4500 mg kg^{-1} Cr. The vegetation when analysed contained 1200 mg kg^{-1} of copper, 1600 mg kg^{-1} of chromium and 1400 mg kg^{-1} of arsenic on a dry weight basis (Grant & Dobbs, 1977).

Through the natural weathering of rock, metals are released into the soil. Generally the concentrations of heavy metal released from such weathering processes is low. However some minerals contain elevated concentrations of heavy metals (metal ores). In an experiment unweathered heavy metals represented by four types of ore were added to experimental coniferous forest soil. Young conifers were potted into the prepared soils and the seedlings were then returned to experimental plots within a forest and left for a period of 3-4 years. At the end of the study period the seedlings were harvested and divided into leaf, stem and root fractions and analysed for their heavy metal content. The concentrations of the added metals increased in the leaves, stems and roots of each experimental group of seedling. The lead content was generally higher in stems and roots in amended soil while mercury levels were up to 10 fold higher in trees grown in contaminated soil. The experiment demonstrates that over a period of time soil weathering processes release soluble heavy metals which are accumulated in plant biomass (King et al., 1985).

Kelly et al. (1979) designed an experiment to evaluate the growth and heavy metal uptake of various tree species in soil treated with increasing concentrations of cadmium. The natural soil contained cadmium, lead, copper and zinc concentrations of 0.6, 11.4, 2 and 20.6 mg kg^{-1} respectively which was then amended with additions of cadmium chloride to give cadmium concentrations of 0, 15 and 100 mg kg^{-1}. The study trees including white pine (*Pinus strobus L.*), loblolly pine (*P. taeda* L.), yellow poplar (*Liriodendron tulipifera* L.), yellow birch (*Betula alleghaniensis* Britt.) and choke cherry (*Prunus virginiana* L.) were germinated from seed in the cadmium contaminated soil. All study species were found to exhibit increased cadmium uptake into roots and shoots in response to increasing soil cadmium concentrations. Likewise all species exhibited decreased root and shoot biomass production with increasing cadmium addition although the reduction was not statistically significant in every case. The 100 mg kg^{-1} soil cadmium treatment resulted in cadmium concentrations within study species of between 28 and 117 mg kg^{-1}. The cadmium concentration in the control species varied between 2.1 and 7.2 mg kg^{-1}.

Heavy metal accumulation in vegetation was studied by growing various species of plant in sewage sludge amended with metal salts. The vegetation including poplar species were grown in pots of de-watered sewage sludge (pH 6.7) which were amended by the addition of cadmium, chromium, copper, nickel, lead and zinc acetates which were added either singly or in combination. Cadmium proved to be the most toxic of the investigated metals at treatment levels of between 100 and 300 mg kg^{-1}. At cadmium treatments of 300 mg kg^{-1} poplar growth was reduced and the maximum leaf concentration was 40 mg kg^{-1} cadmium. Nickel significantly decreased dry matter production at doses of 250, 500 and 700 mg kg^{-1}. Copper was toxic at treatment levels between 1000 and 1500 mg kg^{-1} while zinc was toxic to grass at 3000 mg kg^{-1}. Chromium and lead treatments of between 1000 and 3000 mg kg^{-1} had no significant detrimental effect on plant growth. The toxic threshold concentrations of metals applied in combination was generally lower than for the metal added singly and critical foliage metal concentrations were lower for metals

applied in combination. The foliage concentrations of cadmium, nickel and zinc were correlated with soil metals concentrations. No such correlation was noted for copper, chromium and lead. The order of metal toxicity recorded as a 20% reduction in yield was Cd < Ni < Cu < Zn < Cr and Pb (Smilde, 1981).

Sewage sludge is known to contain elevated concentrations of heavy metals and concerns have been raised over the over application of sewage to agricultural soils. In an experiment at a tree nursery, digested sewage sludge was applied to test soils at rates of 0, 50, 112, 224 and 448 dry metric tons per hectare. The heavy metal content of the sludge was 2154 mg Zn kg^{-1}, 517 mg Cu kg^{-1}, 13.9 mg Cd kg^{-1} and 69 mg Ni kg^{-1}. The experiment studied the growth and heavy metal uptake by pearl millet and various tree species growing in the sludge contaminated soil. The study found that rather than having a detrimental effect, the seedlings growing in the sludge amended soil grew taller, appeared more vigorous and retained their foliage longer than the control seedlings. The pearl millet tissue cadmium levels increased from 0.4 to 1.62 mg kg^{-1} with increasing additions of sewage sludge, all other metal levels in tree seedlings were low and at normal background levels for all treatments (Korcak et al., 1979).

Three year old spruce (*Picea abies*) seedlings were planted in pots of homogenised sand, peat and forest soil amended with treatments of zinc and cadmium. Following treatment annual xylem growth rings were significantly narrower and growth reductions were more pronounced especially in the second year and root development was impeded at moderate metal concentrations. Cadmium and zinc concentrations in stem wood and needles correlated with substrate treatment levels. Hagemeyer et al. (1994) concluded that heavy metals could cause growth reductions in economic forests.

Two year old silver maples (*Acer saccharinum L.*) were grown for two weeks in sand culture amended with 5 and 20 ppm cadmium chloride. Leaves displayed signs of chlorosis and decreased iron content although zinc content was unaffected. Foliage in stages of active growth was more sensitive to the applied cadmium. At treatment

levels of 20 ppm Smith & Brennan (1984) reported a reduction in stem height, leaf number and significant root damage in the study trees.

Breckle & Kahle (1992) studied the effects of root applied lead and cadmium in beech seedlings (*Fagus sylvatica L.*) with regard to various growth, transpiration and nutrient uptake parameters. Treatments of 20 ppm lead and 1 ppm cadmium reduced the levels of K, Ca, Mg, Fe, Mn and Zn in roots and leaves. A significant reduction in leaf area was noted at foliage lead and cadmium concentrations of 6 and 0.3 ppm respectively. The study concluded that lead levels in the studied acidified forest soils were high enough to influence germination, growth and mineral nutrition of forests.

Rolfe (1973) implemented a study comprising eight tree species, five soil lead treatments (0 to 600 ppm), three soil types and two phosphate applications (0 to 336 kg ha^{-1}). Significant levels of lead were recorded in the leaves, stem and roots of all eight species with all treatments. Uptake of lead increased with increasing soil lead levels and was reduced by up to 50% by soil phosphate applications. The maximum recorded lead levels in biomass was 100, 200 and 1100 ppm in leaves, stems and roots respectively in soil treatments and 700, 1000 and 4000 ppm in nutrient solution studies.

Kahle (1993) studied the response of roots of various tree species to root applied Pb, Cd, Zn, Cu, Ni, Mn and Hg. A reduction in root growth, root elongation, biomass production, root initiation and root hair formation was noted with moderate and excess metal applications.

Burton et al. (1983) studied the interactive effects of cadmium, copper and nickel on Sitka spruce. They recognised that studies using individual metals can lead to a better understanding of the ways in which plants are affected by those individual metals but the findings may not be relevant if more than one contaminating metal is present. It is thought interactive effects may be antagonistic, independent, additive or synergistic. The results showed significant reductions in yields of biomass to copper and cadmium treatments but no effects by nickel were demonstrated. The

conclusion reached was the toxic effects of cadmium and copper may be additive when both metals are present at concentrations which individually reduce yields.

Sharma & Sharma (1993) studied the effect of chromium on the growth and yield of wheat (*Triticum aestivum L.*). The wheat was grown in sand culture supplemented with complete nutrient solution which were amended with sodium dichromate to give chromium concentrations of 0.05, 0.1, 0.25 and 0.5 mM. Reductions were noted in plant height, leaf number and tiller number with increasing additions of chromium. Maximum chromium uptake levels of 191.4, 48.0 and 1271.3 $\mu g\ g^{-1}$ dry weight in leaves, stem and roots was recorded respectively.

Studies on ten plant species belonging to five taxonomic groups (Cruciferae, Cucurbitaceae, Gramineae, Leguminosae and Solanaceae) grown in a sand/soil substrate at two pH levels. The soils were subject to cadmium applications of between 0 and 700 mg kg^{-1} (cadmium chloride). Cadmium accumulation was dependent on families irrespective of soil pH. Japanese cabbage and Chinese radish (*Cruciferae*) were the most tolerant, kidney bean and Soya bean (*Leguminosae*)were least tolerant to cadmium (Kuboi et al., 1987).

Mitchell et al. (1988) studied the effect of soil incorporated copper on the emergence and early growth of three Australian native species and three crop species. Soil copper treatments of 0, 10, 100, 1000 and 2000 mg kg^{-1} (copper sulphate) were prepared in a sandy loam soil. Increasing copper concentrations were recorded to delay seedling emergence and reduce seedling growth.

Turner & Dickinson (1993) collected the seeds and seedlings of sycamore growing at contaminated and uncontaminated sites. The growth of the collected seedlings was studied in both nutrient solution and soil experiments. Copper sulphate was added to the nutrient solutions to create copper concentrations of 0, 0.5 and 1.0 mg l^{-1}, and lead nitrate was added to give lead concentrations of 0, 1, 6 and 12 mg l^{-1}. Phosphate was omitted from the nutrient solutions containing lead to prevent precipitation of lead. Shoot and root growth was inhibited at copper concentrations of 0.5 and 1.0 mg

l^{-1}. The roots and shoots contained higher concentrations of copper when compared with control trees, with the highest concentrations of copper recorded in the roots, followed by the foliage then the stem. Increasing lead concentrations were found to inhibited root growth and the highest concentrations of lead were recorded in the roots, then stem with the lowest concentration in the leaves. Metal tolerance was not generally detected in the sycamore seedlings, however, most seedlings survived for the 3 year study period in the most contaminated soil. A further experiment was setup comprising alternative layers of contaminated and uncontaminated soil placed in discrete layers of contaminated and uncontaminated. The tree seedlings were planted into the surface soil and left to grow. The seedling roots were found to proliferate in the uncontaminated zones and avoid the contaminated zones. This effect was attributed to either the adaptation of tree roots to avoid heavy metal contamination or inhibition of tree roots in contaminated soil.

The growth of *Kandelia candel (L.)* was studied in both pot experiments and hydroponics to investigate the influence of heavy metals and salinity (NaCl) on growth. Zinc solution concentrations of 1,5, 25 and 125 mg l^{-1} were prepared from zinc sulphate and copper solution concentrations of 0.1, 0.5, 1.25, 2.5, 5 and 10 were prepared from copper sulphate. Copper and zinc soil concentrations of 0, 125, 250 and 400 mg kg^{-1} were used in pot experiments. Plant growth decreased with increasing concentrations of both copper, zinc and salinity. However the reduction in growth was less with increasing salinity. Metal accumulation in the roots increased with increasing soil metal concentrations especially in the absence of NaCl and metal accumulation in leaves was only noted with the highest copper and zinc treatments. Similar results were obtained from the pot soil experiment although the results were not so pronounced (Chiu et al., 1995).

Pahlsson (1989) discusses the toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. Zinc is generally reported as the least toxic metal to vascular plants with plant growth little affected at zinc solution concentrations up to 1000 μg l^{-1}. Copper and cadmium have been reported to disrupt metabolic processes and growth at concentrations in excess of 100-200 μg l^{-1} whereas the phytotoxicity of lead is

generally lower. Critical leaf tissue concentrations affecting growth were reported as 200-300 mg kg^{-1} Zn, 15-20 mg kg^{-1} Cu and 8-12 mg kg^{-1} cadmium, although the degree of toxicity is ultimately influenced by metal availability, nutritional status, age of plant, mycorrhizal associations and interaction with other metals in the soil.

Godbold & Hüttermann (1988) studied the inhibition of photosynthesis and transpiration as a result of mercury-induced root damage by growing spruce seedlings in nutrient solutions containing mercuric chloride and methyl mercuric chloride for a period of seven weeks. In general the nature of the added mercury had no effect on the mercury concentration in needles. However, methyl mercury treatments resulted in higher levels of mercury in the roots at low levels but not at treatment concentrations in excess of 1000 nM Hg. Methyl mercury significantly reduced transpiration rates and chlorophyll levels, closing stomata and decreasing carbon dioxide uptake levels. Mercury concentrations in roots exceeded levels in the foliage by a factor of 100. The mercury treatments were also noted to cause a reduction in root dry weight, a decrease in some minerals (Co, Zn and Mn) in roots and reduced moisture contents of needles.

Gussarsson & Jensen (1992) performed uptake and leakage studies to determine the effect of copper and cadmium on K^+ fluxes in birch (*Betula pendula*) roots. Pretreatment of the roots with copper reduced passive K^+ influx more than pretreatment with cadmium. However, both metals increased the efflux of K^+ by a similar amount.

The uptake and transport of lead was studied in perennial ryegrass grown in nutrient solution. Root uptake was assessed by measuring the decrease in lead concentration in the aqueous solution. Lead removal was complete and rapid at solution concentrations of 1 mg l^{-1} and was unaffected by removing shoots or killing roots. Total uptake increased with increasing rates of addition and ranged from 281 to 9969 $\mu g\ g^{-1}$. Between 22.7 and 35% of total uptake reached the shoots over a 7 day period. Measured concentrations in roots were between 5.5 and 5310 ppm while concentrations in shoots were between 0.2 and 58.4 ppm. Jones et al. (1973)

concluded that ryegrass roots accumulate lead and act as a barrier which restricts the movement of lead to above ground biomass and is likely to be an important factor in reducing metal toxicity to vegetation.

The uptake and distribution of inorganic mercury ($HgCl_2$) within higher plants (*Pisum sativum* and *Mentha spicata*) was studied in nutrient solutions. The plants tolerated mercury concentrations of 1 mg l^{-1} of solution but physiological and biochemical processes were affected at 5 and 10 mg l^{-1}. Mercury uptake increased with increasing external concentrations, accumulation in roots was linear (log-log) and uptake into shoots was two phase. The major proportion of Hg was tightly bound to the cell wall and was not removed by ethanol or hydrochloric acid (Beauford et al., 1977).

Vascular decolouration is an observed early symptom of metal toxicity in white beans (*Phaseolus vulgaris L.)*. Ten day old white beans seedlings were transplanted and grown in nutrient solution containing 200μM zinc (zinc sulphate) for periods of 24 and 48 hours. Reddish brown patches were noted along leaf veins in both treatments, more patches were evident in the 48 hour treatment. Structural inspections showed modifications of the xylem vessels including gelation of pit membranes, an unusual coating on the lumen surface and deposition of an electron-dense material in the secondary wall. The altered pit layer and coating tested positive for lipid while the electron dense material tested positive for phenolic compounds (Robb et al., 1980).

The accumulation of cadmium was studied with respect to organic matter content and cation exchange capacity (CEC) of the soil. Cadmium accumulation in oat shoots (*Avena sativa L.*) decreased with increasing CEC, however, organic matter content did not influence cadmium accumulation. Haghiri (1974) concluded that cadmium retained on organic matter is predominantly through cation exchange rather than chelation.

Arduini et al. (1995) studied the effects of copper on root growth and morphology of stone pine and maritime pine in nutrient solution. The studies found 5μM copper solution completely inhibited root growth within 3 days and membrane damage occurred after 10 days exposure. Copper treatments also caused an increase in root diameters. The study concluded that cell elongation was more sensitive to copper than cell division.

Rapidly dividing cell suspensions of *Datura innoxia* were found to remove certain toxic metals from nutrient and waste solutions. The micronutrients were found to bind to different components of primary cell wall. The process could be adapted for bioremediation of nutrient and waste streams (Jackson et al., 1993).

Where possible in earlier and subsequent sections the literature reviewed has been grouped under similar fields of study. This section discusses previous studies in which vegetation has been exposed to heavy metals and some of the resulting effects have been studied. The source of the applied heavy metals are varied and include atmospheric deposition, sewage sludge, sand culture, hydroponics and soil culture. The effects studied have included metal uptake, vegetation survival, inhibition of photosynthesis, metal toxicity, root morphology and growth. It should be noted that the information is invaluable and has been considered in the design of future experimental work. The research published by Grant & Dobbs (1977), Kelly et al. (1979), Smilde (1981), Kuboi et al. (1987) and Mitchell et al. (1988) were consulted and some of the information provided has been implemented in the design of the pot experiment including information on the relative toxicities of various heavy metals. Reference was made to work by Arduini et al. (1995), Beauford et al. (1977), Chiu et al. (1995) and Robb et al. (1980) in the design of the hydroponic experiments.

Future reference will be made to the literature reviewed in this section for the experimental design and setup of both the pot experiments and hydroponics (Chapter 4 and 5).

1.3.13 Energy Generation

As discussed the uptake of nutrients and heavy metals by vegetation is a complex process which is influenced by the nature of the soil, the concentration and type of pollution, the species of vegetation and other environmental factors. However previous work has confirmed that vegetation growing on contaminated soil can accumulate heavy metals. The use of trees to remove and accumulate heavy metals from contaminated soil may be beneficial in remediating derelict land. In addition the timber produced could be harvested and sold as timber and fuel for the generation of electricity. Short rotation coppice has been described as forestry grown on agricultural land using horticultural techniques and the most productive coppice woodland identified from field studies are hybrid willow or poplar clones which are harvested on a 2-3 year cycle. The coppice stool life is believed to be approximately 30 years which allows for approximately 10 harvests before replanting will be necessary. Superior yielding varieties of willow (Sennerby-Forsse, 1994) can produce up to 18 tonnes of dry matter annually per hectare and mechanised harvesting procedures (Deboys, 1994) have been developed to increase labour productivity and ease of harvest.

The requirement for sources of renewable energy has been apparent since the 1970's when there were concerns over high oil prices and the finite nature of fossil fuels. In recent times further concerns have been raised over the environmental impact of carbon dioxide gas created by the burning of fossil fuels. In 1994 'renewables' represented only 1% of the fuel in the £26 billion UK generation market. In this regard the Government Renewable Advisory Group speculated that if 16% of the agricultural land was turned over to coppice woodland and the produce was used to create energy the energy created could account for 22% of UK electricity consumption (Piper, 1994).

The accelerating factor in the adoption of wood as a renewable energy source will be driven by economic and technical motivation. Pardé (1980) suggests that the use of existing forest waste including discarded branches and foliage and off-cuts could

save some 20 million tons of oil each year if the waste was burned instead of oil to produce energy.

Maximising the efficient production of energy biomass is the major goal in achieving greater energy independence. The main factors which are likely to influence productivity include:

- Bio-ecological factors (site, tree species, photosynthetic activity, soil moisture and nutrient availability)
- Genetic improvement (hybrids, clones, breeding for biomass productivity and energy efficiency)
- Cultural treatments (soil preparation, planting stock, planting spacing, weed control, fertilisation, irrigation, pest control and re-sprouting)

In summary the use of intensively managed genetically improved coppice woodland could make significant contributions to energy supply. Wood biomass is also recognised as a safer and healthier form of energy when compared with nuclear or coal (Anderson et al., 1983).

The markets for coppice woodland products are relatively undeveloped in Britain but field trials have identified timber produced by coppice woodland as a potential feedstock for the generation of electrical energy. The benefits of planting coppice woodland as a source of renewable energy on derelict land including former mining wastes and areas of mineral extraction has been discussed (Pearce, 1995).

The estimated establishment costs for coppice woodland are approximately £2000/ha which includes for a planting density of 10,000 willow cuttings/ha and crop maintenance including preparing soil and controlling surface vegetation (Pile, 1995).

The use of trees has an economic benefit over other types of vegetation where the biomass produced has no value. Piper (1994) speculated that the wood chips may have an approximate harvest value of £20.00 per ton. Other economic benefits will include employment both in the management and harvesting of the woodlands and

when planted on derelict land the trees will also help to vegetate and screen the site. Coppice woodland can also be cleared quickly from a site should a developer wish to use the site.

1.3.14 Hyperaccumulation

A number of authors have discussed the benefits of developing strategies using plants to remediate soil and ground contamination (Stomp et al., 1993, Cunningham & Berti, 1993). Perennial species like trees have an extensive root mass and high transpiration rates which are beneficial for waste water clean-up and site stabilisation.

A small number of wild plants which grow on metal contaminated soil have been shown to accumulate large quantities of heavy metals in roots and shoots. These plants could be exploited for soil reclamation provided they are easily cultivated and have a high biomass production. One such plant which has been identified is *Brassica juncea (L.) Czern* which has the ability to accumulate Pb, Cr^{6+}, Cd, Ni, Zn and Cu. It can also yield up to 18 tons ha^{-1} of biomass per year. Lead contents have been recorded in shoots and roots of 35.4 and 108.3 mg kg^{-1} dry weight. Based on these concentrations a crop could remove 630 kg of lead ha^{-1} $year^{-1}$ in harvested shoots and the removal rate could be higher if roots were harvested as well (Kumar et al., 1995).

One of the largest groups of 'metal hyper-accumulators' is found in the genus *Alyssum* which can accumulate nickel up to concentrations in the order of 3% leaf dry weight. However the means by which these plants survive with such high internal nickel concentrations is poorly understood. In an experiment histidine supplied to study plants was found to increase nickel tolerance and nickel transport to the shoots. The increased production of histidine is thought to aid nickel accumulation due to the complexation of nickel with histidine which improves nickel transport in xylem and therefore accumulation in the shoot (Krämer et al., 1996).

Brown et al. (1994) compared two metallophytes (*Thlaspi caerulescens* and *Silene vulgaris*) to tomato (*Lycopersicon esculentum*) in a pot study to assess the uptake of copper and cadmium in relation to soil pH. The study soils were collected in the vicinity of an old zinc smelter and chemical analysis identified soil metal concentrations of 48 000, 4100 and 200 mg kg^{-1} of zinc, 1020, 37.4 and 35.2 mg kg^{-1} of cadmium, the soil pH was either 5.06 or 7.04. *Thlaspi* showed greater tolerance and accumulated zinc and cadmium to a maximum concentration of 18455 and 1020 mg kg^{-1} dry weight in shoots respectively without any yield reduction. Selective extraction of the soil indicated that zinc levels in biomass correlated well with the concentration of zinc extracted with neutral salts.

A similar experiment undertaken in nutrient solutions using zinc and cadmium solutions at 50:1 molar ratio. Again *Thlaspi caerulesens* displayed greater tolerance of the zinc/cadmium treatment than the other study species. The shoot concentrations of zinc and cadmium were 33600 and 1140 mg kg^{-1} dry weight respectively. These uptake levels indicate the effective translocation of zinc and cadmium from solution to shoots and suggest *Thlaspi* may be a suitable species for the cost effective remediation of zinc contaminated soil (Brown et al., 1995).

The removal of metals from solution is useful process for treating storm waters, acid mine drainage and agricultural runoff. The use of plants to remove metals from liquid waste has been termed 'rhizofiltration' and is considered to be an important cleanup technology.

The bioaccumulation of indium (In) and dysprosium (Dy) by red alder roots was tested in a small stream in Oregon. The elements were introduced into the stream as non-radioactive tracers. Non chelated In was sorbed by roots up to concentrations of 1.7 mg kg^{-1} dry weight of roots. The chelated In (In-DTPA) sorption rate was greater by a factor of 2. Non chelated Dy was sorbed up to a concentration of 29 mg kg^{-1} dry weight while chelated Dy was sorbed less by a factor of 8 (Knaus & El-Fawaris, 1981).

The roots of Indian mustard (*Brassica juncea L.*), sunflower (*Helianthus annuus L.*) and various grasses were found to effectively remove Cu^{2+}, Cd^{2+}, Cr^{6+}, Ni^{2+}, Pb^{2+} and Zn^{2+} from aqueous solution. *B. juncea* accumulated the metals by between 131 and 563 fold on a dry weight basis above the initial solution concentrations. Dried roots and other dead tissue including wood fibre and melon husk were found to be much less effective in removing lead from solution (Dushenkov et al., 1995).

These studies have shown that metallophytes/metal accumulators can remove significant quantities of metal from soils and nutrient solutions and are therefore well suited for use in remediation projects. However it should be noted that some metal accumulators may not be suitable due to small size, difficulty in harvesting, high cost of seeds and lack of commercial value in the end crop. Plants which accumulate high concentrations of toxic metals could also be difficult to dispose of while in some situations it may be economic to recover the metals from either the vegetation or ash after combustion.

1.4 BEHAVIOUR OF METALS IN SOIL

1.4.1 Background

An important factor which has so far been overlooked when considering phytoremediation of contaminated soil is the soil material and the chemical form of the heavy metals present in the soil. Soil is a very complex matrix which is made up of many different components including weathered minerals, organic matter, microorganisms and water. No two soils are completely identical and the behaviour of heavy metals and nutrients in soil involves many complex processes.

In the natural environment soils develop from the chemical alteration and decomposition of the mineral particles of rock and/or superficial deposits which cover the earth's surface. Rock is defined as inorganic mineral material which can be

hard or loose and unconsolidated in the case of gravel, sand or clay (Wild, 1988). The decomposition of rock is not sufficient to distinguish the material as soil. Soil is generally only generated when plants begin to grow and organic remains are incorporated with the decomposed rock. The decomposition of rock can be by physical disintegration without change in chemical composition or by physical forces which include grinding forces, expansion forces, growth pressures, water pressures and wetting and drying cycles. The rock can also be subject to chemical weathering where water, dissolved salts and acids are the important chemical agents which aid decomposition. The mineral weathering processes is characterized by the processes of dissolution, hydration, hydrolysis, oxidation, reduction and carbonation.

The cycle of soil formation requires plants to colonise and grow on the weathered rock material, the plants take up soluble minerals from the weathered rock and use them as nutrients for growth. These minerals are returned to the soil through leaf drip and decomposition of the plant material on death of the plant. Soil animals which feed on dead vegetation also aid development of weathered rock into soil by incorporating organic matter.

When reference is made to heavy metals in the context of contaminated land the definition is generally directed towards those metals which are considered to be harmful to human health, not to mention mammals, fauna and aquatic organisms. In chemical terms heavy metals are those metals which have a specific gravity in excess of 4.5 g cm^{-3}. Metals are generally thought of as something with a bright metallic appearance, good electrical conductivity and high thermal conductivity, however, in nature only a few metals are found as pure elements, the most obvious being the precious metals of silver, gold and platinum. In the soil and rock environment most elements exist as oxides, sulphates, sulphides or carbonates and as carbonates, phosphates and organo-metals in living organisms (Wild, 1988).

In natural soils which have not been subject to industrial pollution the residual heavy metal concentration in the soil is derived from the weathering of parent rock material. Generally the heavy metal input to soil from in-situ weathering of parent

rock is low and only likely to produce potentially toxic metal concentrations locally in areas of heavy metal rich deposits. Such metal rich soils are often colonized by specialised flora including 'hyperaccumulator' species.

Industrial contaminated spoil mounds can arise from the mining and extraction of heavy metal rich minerals, following extraction of the target element the material becomes a waste byproduct which has no further value. These wastes often contain elevated concentrations of one or more heavy metal and are deposited as waste materials in mine tailings and spoil mounds. Heavy metals can also accumulate in natural soils which would otherwise have low heavy metal contents through industrial activities, atmospheric emissions and spillages of heavy metal solutions and liqueurs.

It is important to remember whatever the form or chemical nature of the metal in the soil, soil water acts as the transport medium of heavy metals and solutes in the soil. In temperate areas of the world the net flux of soil water is downwards towards the groundwater table and solutes in the soil water are leached from the topsoil. These lost solutes are replaced as a consequence of the weathering process, plant leaching or aerial deposition. The mobility of ions will depend on the percentage water saturation and velocity of water movement in the soil, redox potential, soil pH, interaction with solution constituents (ligands) and properties of the soil matrix. It should be noted only a fraction of the total metal in the soil will be in solution at a given time, the percentage depends on the chemical properties of the element and the prevailing environmental conditions. In summary the fate of heavy metals in soil will be determined by the environmental conditions, the initial chemical form of the metal and the types of animals and plants in the system.

To understand the mobility of metal ions in solution we must understand the partitioning of metal ions between solid and liquid phases. Solid phases in the soil which carry an excess of negative charge are responsible for removing metal ions from solution. The electrical attraction between opposing forces retains positive ions at negative sites which form at the surfaces of silicate clays and surface functional

groups of soil organic matter. These permanent charge sites of silicate clay minerals and pH dependent charge sites of soil organic matter can retain metal cations by non-specific electrostatic attraction. Heavy metals such as copper, lead and cadmium compete with the more abundant soil cations (sodium, magnesium and calcium) for cation exchange sites. For this reason, strong partitioning of heavy metals onto cation exchange sites is not normally observed. Therefore many heavy metals are specifically adsorbed, or chemisorbed onto amorphous oxides of aluminium, iron and manganese and soil organic matter. For most heavy metals retention is by specific sorption by iron and manganese oxides and by soil organic matter rather than by non-specific cation exchange except for zinc and cadmium. The rates and direction of these processes are strongly influenced by the acidity and redox potential of the soil and will be discussed later. The concentration of metals on the soil solid phase are generally larger than the concentrations in the solution phase and in this regard soil retention processes tend to be much more important than metal leaching.

The main processes associated with the retention and mobility of metals in soils are listed below and are discussed by Wild (1988):

- Solubility
- Exchange on cation exchange sites
- Specific Adsorption and chemisorption
- Precipitation
- Tertiary Complexes
- Organic Complexation and Chelation
- Immobilization by soil organisms
- Mobility, leaching and transport
- Uptake by plants

1.4.2 Solubility

The principle factors influencing the solubility and concentration of heavy metals in the soil solution is the chemical form of the heavy metal, soil pH, soluble organic matter and soil redox potential.

Chemical Form

Once in solution, heavy metal ions (simple or complex) exhibit typical exchange behaviour on silicate clay minerals in the soil. The strength of the metal bonding is dependent on ionic charge and hydration characteristics of the metal ion. The ionic potential of elements is the ratio of ionic charge to ionic radius and is a useful indication of the relative solubility of an ion. An element with low ionic potential is generally more soluble than an element with a higher ionic potential. The alkali cations, sodium and potassium have ionic ratios lower than 30 and are soluble, are easily weathered to form hydrated cations and are easily leached from soil. Ions of the transition metals and heavy metals have ionic ratios greater than 30 and have intermediate ionic potentials, are not very soluble and when weathered they precipitate as oxyhydroxides.

pH and Redox Potential

One of the most important factors controlling metal solubility in soils is acidity. The leaching of mineral cations from temperate soils without replenishment, acid precipitation, organic matter breakdown and root ion uptake can all contribute to local acidification in the soil. Generally heavy metals in the soil become more mobile under acidic, oxidizing conditions and are retained strongly in alkaline, reducing soil conditions.

Studies have shown that Zn, Cd, Cu and Pb are more soluble at soil pH 4 to 5 rather than soil pH 5 to 7 (Brümmer et al., 1983). Robb et al. (1980) reported increasing uptake of Zn, Fe & Mn by French beans with increasing acidity of the soil rhizosphere.

Organic Ligands

Low molecular weight organic molecules which are soluble in the soil solution can form organ-metal complexes with heavy metals. These complexes are soluble and mobile in the soil solution and can be taken up by plants. In this regard soils which contain high concentrations of organic compounds may contain higher concentrations of soluble heavy metals.

1.4.3 Exchange on Cation Exchange Sites

The exchange of metal ions occurs on the permanent charge sites of silicate clay minerals and pH dependent charge sites of soil organic matter by the process of non-specific electrostatic attraction. The effect of this charge which is the product of weathering and soil development is responsible for the behaviour of plant nutrients and the capability of a soil to adsorb potential pollutants. An equilibrium exists between ions in the soil solution and those held on charged surfaces and the extent of cation attraction and anion repulsion is dictated by the surface density of the negative charge.

1.4.4 Specific Adsorption and Chemisorption

The non-exchangeable complexing and adsorption of free aqueous ions, ion pairs and complex species is widely described as adsorption, chemisorption or sorption and takes place on the surfaces of silica, amorphous aluminosilicates, aluminium hydroxides and most importantly the surfaces of iron and manganese oxides. Metal adsorption processes in soil are very complex and include a large number of chemical species, adsorbing surfaces, time dependent steps and bonding mechanisms.

The sorption process is believed to be two-step, an initially rapid first step which represents adsorption onto highly accessible sites followed by a second, slower sorption process of co-precipitation with iron and aluminium oxides (Wild, 1988). Metal sorption/adsorption is determined by three different steps; surface adsorption followed by diffusion of the metal into the mineral structure and fixation at positions within the mineral structure.

The specific adsorption of heavy metals by soil minerals is relative to metal ion hydrolysis. As the ability of metals to form hydroxy complexes increases so does their likelihood of specific adsorption. The order of heavy metal hydrolysis has been reported as Cd< Ni <Co < Zn << Cu < Pb < Hg. Which is the same order for increasing specific adsorption (Brümmer et al., 1986).

Oxide-metal bonding is not entirely electrostatic, since it is not possible to predict the bonding order based on ionic potential and some covalent interaction is apparent. Transition metals classified as 'hard' bond to oxides more strongly than 'soft' transition metals. However, 'soft' non-transition metals like lead are preferred over 'harder' non-transition metals (cadmium and magnesium), while zinc is reported to display intermediate behaviour (McBride, 1984).

Apart from the types and amounts of soil colloids (clay minerals, soil oxides and organic matter), metal sorption in the soil is influenced by: soil pH, ionic concentrations in the soil solution, metal cation concentration, the presence of competing metal cations and the presence of organic and inorganic ligands. Metal sorption is reduced by increasing soil acidity, however, some divalent transition and heavy metal cations can be adsorbed by iron and aluminium hydrous gels at low pH and are important for metal retention in acid soils.

Competing ions such as Co, Ni and Zn have more of an influence on Cd sorption than do Cr, Cu and Pb. Cadmium sorption in a soil is mainly by cation exchange and Co, Ni and Zn are recognised as better competitors for electrostatic, non-specific sites than Cu and Pb which adsorb specifically on Fe/Mn oxides and organic matter

(Christensen, 1987). In this regard oxides and organic fractions in the soil adsorb Pb, Cu and Zn in preference to Cd, Ni and Co. Lead and copper are more strongly retained by soil colloids (soil oxides and organic fractions) than cadmium, zinc and nickel. However, cadmium and zinc have more cation exchange ability on layer silicates.

An important issue when assessing the concentration and uptake of heavy metals in contaminated soil is whether adsorbed metals can subsequently become desorbed and available for uptake. Ion exchange studies indicate metal sorption on iron and aluminium oxides occurs by the formation of inner sphere complexes. Adsorbed metals are only likely to desorb by exchanging with H^+ ions or metal cations which have a specific affinity for the oxide. Metal desorption is reported to differ from patterns of metal sorption. McLaren et al. (1986) reported that substantial amounts of cobalt could be desorbed from montmorillonite clay, humic acid and soil oxide, however, the shape of the desorption isotherm differed markedly from that of the adsorption isotherm.

A summary of the retention pattern for most trace metals in relation to soil pH can be summarized as follows:

- at low soil pH (2-4) cation exchange ability is dependent on valence and ionic size
- at intermediate soil pH (4-6) metal ions form soluble hydroxy species which are adsorbed on clay surfaces
- and when soil pH exceeds (5) precipitation processes dominate.

The effect of soil pH on metal adsorption is strong for Cd, Zn and Pb, but less so for Cu (Wild, 1988). In summary cadmium and zinc are generally considered to be more mobile than copper and lead in soil. Since cadmium and zinc ions tend to be sorbed by cation exchange and can be exchanged by competing ions in the soil solution they are likely to be more mobile in acid soils and soils with a high organic matter content while Al/Fe oxides will preferentially retain copper and lead .

1.4.5 Precipitation

Both adsorption and precipitation of metals can occur at mineral surfaces, however, it is often difficult to distinguish between the two. Manganese oxides promote the oxidation of manganese and iron may promote co-precipitation rather than adsorption.

Adsorption reactions at low solution cation concentrations is believed to be 'true' adsorption, however, at higher solution concentrations, particularly with copper, there is a decrease in soil pH. This has been attributed to the formation of copper compounds including the precipitation of $Cu(OH)_2$ (Papadopoulos, 1985).

1.4.6 Organic Complexation and Chelation

Organic complexation of metals in soils and waters is thought to be one of the most important factors governing solubility and bioavailability of metals in the soil-plant environment. The range and variety of organic compounds in the environment is immense, however, all organic compounds can be grouped in to three main classes which can form metal complexes;

1. naturally occurring soil organic molecules of known structure and chemical properties; aliphatic acids, polysaccharides, amino acids and polyphenols.
2. anthropogenically derived organic chemicals from agriculture, industrial and urban activities.
3. humic and fulvic acids which accumulate in soil but have no detailed structures

The major input of soil organic matter is from the decomposition of plant residues, however, the structure of soil organic matter is generally of no fixed composition due

to the complex decay processes which continually changes the composition of the organic matter. Soil organic matter can be segregated in to three main groups; large molecular weight humin and humic acid compounds, lower molecular weight fulvic acids and a third group which comprises carbohydrates, proteins and amino acids (Kononova, 1966). Humic acids have molecular weights of between 2000-10000 and fulvic acids have molecular weights between 500-2000. Carbohydrates (cellulose, hemicelluloses and polysaccharides) and nitrogen containing compounds (proteins and amino acids) are generally present in the soil in the smallest concentrations because they are the first and easiest to be broken down by soil micro-organisms. Plant protecting compounds (lignin, tannins and waxes) are broken down with difficulty and remain in the soil for longer periods.

The mobility of humic and fulvic acids in soil is dependent on the viscosity in the soil solution, size of the molecule and shape of the molecule. Large organic molecules will be less mobile. Humic acids tend to form insoluble complexes with metal species regardless of the molecular weight of the HA. The addition of an electrolyte to a humic acid solution causes an increase in viscosity and the complexation of metals can influence the size and shape of the organic molecule (Perdue, 1985). Many branched polymer complexes are likely to have smaller frictional surfaces and are therefore more mobile.

Although humic acids have important metal binding capacity their molecular size and configuration means they are generally less mobile and less likely to be leached down the soil profile. Fulvic acids are important for the binding and transportation of toxic metals within the soil solution. The enhanced solubility of toxic metals through organic chelation is responsible for the leaching of contaminants from landfills to surface and ground waters. Metal chelates also influence the bioavailability and transport of toxic metals to plants and can also reduce the concentrations of toxic metal ions in the soil solution.

The major functional sites for metal bonding on soil organic matter are functional groups containing oxygen including carboxyl, phenol, alcohol and carbonyl groups.

Factors which influence complexation of metals with soil organic matter are; the electronic status of the ligand site, associated aliphatic chains, aromatic rings, geometry of the functional site, pH, the ionic strength of the bathing solution and the metal species.

The importance of COOH groups on humic and fulvic acid for metal complexing was studied using infra-red spectroscopy (Jackson et al., 1993). When the acid carboxyl (COOH) and the phenolic hydroxyl (OH) groups were blocked there was a significant reduction in metal binding during chelation. In this regard oxygen containing ligands appear to be the most important groups for metal binding.

The order of affinity of metal ions for fulvic acid follows the sequence of Fe > Cu > Zn > Mn > Ca > Mg and the affinity sequence of metals for undefined 'humic substances' follows the sequence of Cu > Pb > Zn = Ni > Co > Cd > Mn > Ca > Mg.

Two levels of bonding has been identified between metal ions and organic matter using Electron Spin Resonance (ESR). An inner sphere complex in which the metal ions form a bond of covalent character with HA ligands and outer sphere complexes in which the metal ions are electrostatically attracted to HA functional ligands.

In general terms most divalent metal ions, with the exception of Cu, Pb, Fe and V are bound in outer sphere complexes, with the more tightly bound inner sphere metal ions likely to be in the hydrated condition.

The spreading of sewage sludges on agricultural soils contributes to the concentration of toxic metals with oxygen organo-metallic complexes in agricultural soils. Sewage sludge can consist of up to 60% humic and fulvic acids, is high in protein materials, sulphur containing compounds and phenolic compounds. The amide-N and amide-O site have been identified as important sites of metal binding in sewage sludges (Tadesse et al., 1991). As a result of the metal binding capacity of sludges and the elevated concentrations of toxic metals in sludge, the addition of

sludges can increase the total metal content and water-soluble concentrations of metal ligand complexes in the soil.

1.4.7 Immobilisation by Soil Organisms

Soils can contain a wide range of biological forms varying in size from submicroscopic viruses and bacteriophage particles, through bacteria, actinomycetes, algae, protozoa and fungi, small mites of the meso-fauna to large earthworms. The decomposition of organic matter by soil organisms results in the liberation of soluble nutrients for plant uptake and various organic compounds. Some of the organic compounds released will be chelating agents which will interact with heavy metals in the soil. These chelating agents are also believed to be important in the solubilisation of insoluble minerals and the accelerated weathering of soil minerals.

Soil organisms behave rather like soil organic matter and the decomposition products adsorb and bind with metals in the soil. The adsorption and binding of soil metals effectively removes available toxic metals from the soil solution. However, when the chelating agent is soluble the bound metal will be mobile, this increased metal solubility has been shown with ferric ions and fulvic acids (Perdue, 1985).

1.4.8 Mobility, Leaching and Transport

The leaching of heavy metals from soil constitutes an effective loss of metal from the soil environment and can cause environmental contamination of surface and ground waters. The mobility of soluble metal species present in the soil solution is determined by diffusion and mass flow.

The increased mobility and leaching of aluminium as a result of increasing soil acidity has been studied and proven in forest soils (Keltjens & Van Loenen, 1989). However, further research has also identified increasing mobility and leaching of magnesium, calcium, manganese, zinc and cadmium with regard to increasing soil

acidity. The order of mobility of metal ions with regard to increasing soil acidity is believed to be Al, Mn, Zn (high) > Cd, Co, Cu, Ni, > Pb, V (low) (Hogan & Wotton, 1984). In general increasing soil acidification may be expected to mobilize and enhance leaching of Al, Mn, Zn and Cd and Ni to a lesser extent, however, increasing acidity is unlikely to influence the leaching of Cu and Pb, particularly when they are strongly associated with immobile soil organic matter.

In podzols, large quantities of organic matter released from the surface horizons form organic chelates of aluminium and cadmium which results in the enhanced leaching of cadmium and aluminium down the soil profile. Soil acidity was found to be the major factor influencing the leaching of aluminium while soluble organic matter was the major factor influencing the leaching of cadmium (Berggren, 1992).

1.4.9 Uptake by Plants

In simple terms minerals absorbed by the roots of plants and phytosynthate produced in the leaves are used to produce the compounds necessary for plant growth. The availability of mineral ions in the soil and their transport to and uptake at the root surface is important is determining the growth of plants. The uptake of metals and nutrients by vegetation effectively removes metals from the soil and soil solution. The retention of metals within vegetation can vary between a few months and many years and is dependent on the life cycle and storage mechanisms of the plant.

1.5 SUMMARY

During the review of published literature no previous research was found relating to work undertaken on the phytoremediation/phytoextraction of heavy metals from contaminated soil by coppice woodland. In this regard it has been assumed no previous works have addressed this area of research. Some of the information obtained, however, does provide important information which will be useful for

experimental design purposes. The information includes details of soil metal concentrations and soil nutrient solution concentrations which were found to be toxic to study species. Details of the literature consulted and the chosen soil and solution concentrations will be discussed in the experimental design sections of each relevant experiment.

Positive information has been obtained from the literature including reports by many authors of the accumulation of heavy metals by vegetation growing in heavy metal contaminated soil and nutrient solutions. The levels of uptake reported are generally greater when compared with the uptake of similar control plants grown in non-contaminated solutions. However, it is apparent that the tree species studied have not shown the same level of hyper-accumulation of heavy metals as has been shown by metallophytes. Trees and especially coppice woodland is known to be high yielding and provided metal uptake is reasonable the combination of high biomass yields should compensate for the lower level of metal accumulation. The biomass produced by coppice woodlands can also be harvested and sold for energy generation. This type of energy is renewable and would reduce the generation of carbon dioxide which is a major factor in global warming.

The behaviour of heavy metals in soil has been identified as an important factor when considering the suitability of sites suitable for phytoremediation. Due to the complexity of soil and the complex interactions that occur between soil and heavy metals, trace elements and nutrients it will be necessary to give careful consideration to soil conditions in experimental design. The most important soil characteristics influencing soil metal availability are pH, presence of organic matter, oxides and mineral components of the soil. When heavy metals are added to soils it will be assumed that the metal will be retained mainly within the following pools in the soil.

1. The soil solution (ionic, molecular, chelated and colloidal heavy metal)
2. Readily exchangeable ions (inorganic or organic fractions)
3. adsorption complexes (iron and manganese oxides)
4. Precipitated sesquioxides and insoluble salts

5. Fixed in crystal lattices of secondary clay minerals

CHAPTER 2
EXPERIMENTAL METHODS

2.1 SAMPLING PROCEDURES FOR SOIL

2.1.1 Introduction

The collection of soil requires utilisation of the most appropriate sample collection method to obtain samples representative of the selected study body. When designing the collection and analysis of soil samples it is important that the procedures used are appropriate for identifying the nature of potential contamination both spatially and vertically. Several stages of sampling are considered to provide more flexibility and greater assurance of the final assessment (British Standards Institution (BSI), 1988).

Procedures for surveying and collecting soils are discussed by Bridges (1987), MAFF (1986) and Allen (1974). However variations on the methods given are often used as the method of sampling has to be suitable for the analytical procedures proposed, in this regard appropriate references will be discussed in the relevant Sections.

Before deciding on the appropriate method of sampling, especially contaminated soil assessments, it is important to give careful consideration to the following factors:

- **Sampling Strategy**

The history and environmental setting of a site can be determined by consulting various sources of information including historical Ordnance Survey (OS) sheets, site plans, geological information and previous site investigation records. When some or all of the above information is available, sampling locations can be targeted towards potential 'hot spots' of contaminated soil. In situations where no information is available a formal grid of sampling points can be used (BSI, 1988).

- **Number of sample locations and sample numbers**

The number of sampling points is influenced by; the site area, the distribution and variability of likely contaminants and the degree of confidence in the analytical

results required. In reality no sampling programme can guarantee the identification of every patch of contamination and a pragmatic compromise is essential. Documents produced by British Standards Institute (1988) and Department of the Environment, (1987) suggest between 20 and 50 sampling points per hectare in dubious areas and 5 to 10 per hectare in areas not suspected of significant contamination. Adhering to a regular grid with spacing of between 20 and 50 metres corresponds to between 10 to 25 pits per hectare, respectively.

The number of samples collected from each pit can only be decided by visual inspection and interpretation of individual locations. Generally a minimum of 3 samples per sample point comprising one near the surface (0-200 mm), one from the greatest depth of concern and a random intermediate sample is recommended. In the field the soil profile often contains features which determine the sample pattern and sample numbers.

- **Sampling Depth**

The choice of sampling depth depends on the likelihood of finding contamination, the significance of the hazard and the depth to which the sampling equipment can reach. Sample depth is important when considering possible contamination sources, pathways and potential hazards.

- **Excavation Methods**

Trial pits are one of the simplest means of sampling and are useful for the evaluation of the extent of contamination with depth and area. Trial pits also allow the visual assessment of soil profiles, horizon depths, thickness of contamination and obstructions. The maximum depth of trial pit excavations can vary between two metres for hand pits and six metres using a tracked excavator.

Boreholes are preferred when sampling beyond six metres or when the ground conditions are unstable and pits would require shoring or where in situ gas or ground

water monitoring is required. Boreholes can provide important engineering information especially for deep foundation solutions. However boreholes give less visual information and there is an increased risk of cross contamination between samples due to soil smearing on the drilling equipment.

- **Sample Collection**

The collected soil sample should be a representative sample and it is therefore not advised to bulk samples from different locations or depths. Once a sampling location and depth has been selected it is good practice to obtain a representative sample by combining small sub-samples to give a large composite sample of about 1 kilogram.

It is important the composite sample is held in a suitable container which prevents the loss or degradation of any chemical determinand or cross contamination of the sample.

2.1.2 Sampling 'Field' Soil

Introduction

The sampling recommendations discussed above were followed wherever possible, however, procedures were amended to address the requirements of the study and site conditions. Further reference has been made to procedures developed during previous research at the University of Glasgow, Mallik (1993) and Khattack (1987).

Reference was made to previous site investigation reports prepared by Glasgow University and where applicable the sampling regime has been amended to recognise the findings of the previous site investigations. A review of the reports identified a number of possible 'study' sites where the level of heavy metal contamination was appropriate for the intended study. A site walkover was undertaken to evaluate the suitability of each site and identify species of trees established within each area. From

these initial evaluations two test sites were selected for the field experiments and will be referred to as Redding and Summerford.

Procedure

Shallow trial pits were excavated by hand at selected positions below the leaf canopy of selected study trees within the study areas at Summerford and Redding. The trial pits were excavated to 0.50 metres depth and two soil samples were collected. Sample 1 was collected from between ground level and 0.25 metres depth and sample 2 was collected from between 0.25 and 0.50 metres depth. The samples were collected into polythene sample bags which were clearly labelled with the site name, depth of sample, tree species and date of collection. In all cases approximately 1 kilogram of soil was collected. The sample bags were tied tightly and taken to the laboratory on the day of collection. In situations where it was not possible to prepare the soil the samples were temporarily stored in a cold room at a temperature of 0-4 ^{0}C. Care was taken to ensure sampling equipment was rigorously cleaned between sampling points to reduce the risk of cross contamination.

2.1.3 Sampling 'Pot' Soil

Introduction

The method used for the collection of soil samples in the field was considered unsuitable for collecting representative soil samples from test pots containing 8 Kg of soil. It was also considered the experimental design and the potential spread of contamination within the pots would have given inaccurate results if only surface samples of soil were collected. The sampling procedure therefore required to take a vertical sample of the soil distributed through the pot to alleviate any error arising from the downward leaching of metals through the soil profile. The chosen sampling method comprised a crude form of 'window' sampler in order to obtain vertical soil cores from test pots.

Procedure

Plastic conjugate piping (20 mm in diameter) was cut into 300 mm lengths. The piping was cleaned in decon and rinsed with deionised water to remove any contamination.

The conjugate piping was forced vertically down through the soil profile of selected test pots. This procedure had the effect of forcing a vertical column of soil into the centre of the pipe. On removal of the pipe the vertical column of soil was lifted clear of the trial pot. The sampled soil was shaken from the sample tube into a labelled polythene sample bag. Five separate soil cores were collected and combined from five replicate metal and tree treatments. The process was repeated for each metal concentration and seedling treatment.

The soil samples were labelled with date of collection, tree species and soil metal concentration details. Samples were stored under refrigerated conditions prior to preparation and analysis.

2.1.4 Soil Preparation

Introduction

Fresh soil is in most instances unsuitable for use in laboratory testing procedures and requires further preparation. The level of preparation and the method of preparation should be chosen to complement the laboratory tests or work which is to be undertaken. Where the soil is to be utilised for microbiological studies the soil should be maintained in a natural state while chemical analysis procedures require the soil to be air or oven dry (Mallik, 1993, MAFF, 1986 and Khattack, 1987). When results are expressed as oven dry direct comparisons can be made between results obtained for different soils.

Field soil especially contaminated soils contain various quantities of large particulate material including gravel, wood and metal. These components play little part in a soils chemical properties and can be removed by sieving. Laboratory tests are generally undertaken on sieved samples but it is important to note the size of sieve used, again for the comparison of different samples. A widely accepted sieve size is 2 mm. During soil preparation works it is important to maintain good laboratory practice and clean equipment between samples to prevent cross contamination.

Air dried soil is widely used in laboratory analysis techniques, however it is important to note that air-dry soil is not to constant weight. External factors including temperature, relative humidity and sample drying time all influence the water content of air dried soil and as a result different samples will contain marginally different percentages of water. In this regard a sub-sample of air-dried soil can be oven dried and the loss of weight used to calculate a conversion factor. The calculated conversion factor is specific to that batch of soil and can be used to convert the weight of air-dry soil to the comparable weight of oven dried.

In some instances it is preferable to use natural soil, this is especially relevant when the soil is to undergo microbiological tests as any drying process will reduce microbial numbers. Fresh soil should be stored under refrigerated conditions and disposed of after a set time period has elapsed. The presence of large soil components including gravel and other large particles contribute little to the chemical and biological behaviour of soil should be removed where possible. Due to the cohesive properties of moist soil sieving through a 2 mm sieve is almost impossible unless the soil is very sandy and therefore fresh soil is generally sieved through a 4 mm sieve. Again it is important to identify the chosen method of sieving.

- **Preparation of Fresh Soil**

'Fresh Soil' was obtained by sieving freshly collected 'field' soil through a 4 mm stainless steel sieve to remove large gravel, wood and other debris. The sieved soil was retained in a clean, labelled polythene sample bag and stored refrigerated.

- **Preparation of Air-Dry Soil**

Clean, heavy duty, polythene sheeting was laid out over the top of laboratory work benches. Approximately 0.5 kg of the sampled soil was spread over the sheeting to a depth of approximately 1 cm. Large soil aggregates were broken and large stones and other debris removed from the soil by hand. The soil was left open to the atmosphere for between 2 and 4 days at ambient room temperatures.

After the required drying period the soil was sieved through a 2 mm stainless steel sieve. For hygiene reasons the sieving was undertaken in the fume cupboard to prevent dust contamination of the laboratory environment.

The sieved soils were stored in labelled self-seal polythene bags.

- **Preparation of Oven Dry Soil**

Small, clean vitreosil basins were dried to constant weight in an oven at 110 ^{0}C for a period of 24 hours. On removal from the oven the basins were cooled in a desiccator to prevent the re-adsorption of moisture. When cool the basins were weighed and the dry weight of the basin noted. Approximately 10g of 2 mm sieved air-dry soil was weighed accurately into each basin and the weight of soil noted. The basins and soil were returned to the oven and dried to constant weight at 110 ^{0}C over a period of 48 hours. After drying, the basins and soil were removed from the oven, cooled in a desiccator and re-weighed. The weights recorded prior to and after drying can be

inserted into the calculation given below in order to calculate the conversion factor for that soil.

Calculations

$$\textit{Conversion Factor} = \frac{\textit{total oven dry weight - weight. of basin}}{\textit{air dry weight - weight of basin}}$$

2.2. SAMPLING PROCEDURES FOR BIOMASS

2.2.1 Introduction

As with soil sampling, biomass sample collection requires utilisation of the most appropriate sampling method to obtain samples representative of the selected study body. Reference has been made to information published by; Allen (1974), Tadesse et al. (1991), Hoffmann & Lieser (1987) and Moffat and McNeill (1994) with respect to collection procedures for plant biomass. Particular attention has been given to the collection of woody samples.

In theory the sub-sample analysed should have exactly the same average composition as the total sample. Field sampling is the most susceptible to interference and is therefore the greatest source of error. Representative sampling of the total population requires the sampling of heterogeneously distributed individuals. With trees the sampling has to be representative throughout the depth and height of the tree or forest (Moffat and McNeill, 1994).

The heterogeneity of populations is regulated by time induced changes which are influenced by the seasonally alternating increases in biomass, by differences in the balance of content substances due to age and by classic abiotic factors such as soil, light, precipitation and wind. Also the genetic differences between individuals may be responsible for differences in element distribution (Hoffmann & Lieser, 1987).

The nutrient content of different parts of plants vary considerably. In general the tissues richest in nutrients are those where metabolic activity is the highest and include shoot apices, leaves and younger tissue. Wood tissue generally has lower levels of nutrients but due to the overall volume of this fraction in mature woodlands a considerable fraction of the total elemental fraction is present in the wood (Tadesse et al., 1991. For woody species, sampling the current years growth of leaves and/or shoots provides the best indicator of nutrient status (Allen, 1974).

External climatic factors influence the nutrient status of vegetation. Nutrient concentrations within tree foliage differ between sun and shade sides and with height within the crown. Daily and diurnal variations result from the transfer of carbohydrate compounds daily within the tree. Seasonal changes result from the movement of nutrients into components during growth and the reverse when senescence is approaching (Hoffmann & Lieser, 1987).

In deciduous leaves as discussed by Fromm et al. (1987) and Wyttenbach & Tobler (1988) elements such as N, P, K & S show a peak in early spring, remain steady in summer and decline in autumn. Ca, Si & B show little initial change then rise more sharply as autumn approaches. Fe, Al, Zn, Mn, show little change initially before increasing to an early autumn peak with a sharp fall later. The total dry weight increases to a steady maximum from July to September the falls in the autumn. In conifer needles the concentrations of many elements tend to increase during the year, however, needles of different ages however show different seasonal patterns.

Variations in the nutrient status of plants is not solely influenced by climatic factors. Differences in the element composition may occur between individual plants of the same species even within a closely limited stand caused by heterogeneous soil. Reported zinc fluctuations to a maximum of 14% have been attributed to different soil types (Markert & Stanbeck, 1988).

It is important to remember the analysis of a single plant specimen only reflects a snap shot, illustrating the quantity of some element in some plant at some time. Differences in element concentration in and between individual plant species are of a varied nature and it is difficult to predict metal contents in vegetation. In this regard the elemental content of biomass can only be determined experimentally.

Moffat and McNeill (1994) report that foliage sampling should be restricted to stands of 4 metres or less in height and works best when designed to examine specific problems. The main use of foliage sampling is to determine nutrient status and assess the fertiliser requirement of forests and woodlands.

The most appropriate time to sample deciduous conifers and broad leaf trees is in late July or August, after shoot growth is complete but before needles or leaves begin to change colour. Conifer foliage can be collected at any time of the day from the first week in October to the end of the second week in November. Sampling should be avoided after periods of prolonged rain as the rain can leach nutrients from the biomass.

Although the behaviour and distribution of heavy metals will vary for individual elements there are a number of sampling principles which should be considered when designing a sampling strategy.

When developing a sampling strategy it is important to determine whether random or systematic sampling is the most appropriate method:

- **Random Sampling:** random sampling defines the random collection of individual specimens from a total parent population e.g. spruce needles from a spruce forest to determine average calcium content.

- **Systematic Sampling:** the collection of birch leaves from various heights to determine calcium distribution as a function of tree height. (Systemic sampling mainly refers to problems of changeable values as a function of space and time.)

In theory the aim of any sampling program should address to the following principles:

- a sample taken from a population should have exactly the same chemical composition as the original population.
- the probability of being an individual taken from a total population must be equal for each individual.
- the greater the degree of dispersion of individuals and total number of individuals the greater the effort required to sample.

Once a sample has been collected there are a few basic rules to prevent loss or contamination of the sample. Sampling devices and sample containers should be cleaned thoroughly to avoid the transfer of contamination. The sample container should be of an appropriate material to prevent the loss of potential determinands through volatilisation, wall adsorption and deterioration of the sample. Where possible it is recommended that stainless steel cutting devices and clean sample containers are used.

Another important sampling consideration is whether or not the collected sample requires cleaning prior to analysis. Cleaning should always be considered in situations where there is likely to be a high input of dust, flue gas of similar in the study area. Foliage cleaning is also recommended in plant uptake studies when the results of nutrient uptake values from soil would be biased if the plant surface was contaminated by airborne particles. A suitable washing agent can be described as the most efficient washing agent which will not leach metal from the sample. Common washing agents include deionised water, weak acids or detergents. Washing not only applies to above ground tissue, it is also important to wash off soil adhering to roots prior to analysis as a small fraction of rhizosphere soil is likely to bias the analytical data as elemental concentrations in soil, especially contaminated soil, are likely to be more elevated than concentrations in vegetation.

In summary the risk of cross contamination during sampling is high and all reasonable care should be taken to prevent the introduction of contamination which could bias the chemical test results. In situations where contamination by soil is unavoidable Allen (1974) suggests that it is possible to analyse for titanium as titanium is regarded as a component of soil only.

2.2.2 Sampling 'Field' Biomass

The principles of sample collection discussed by Moffat and McNeill (1994) were considered in the design of the sampling programme for 'field study' samples, however some method development was undertaken to address site specific requirements and to recognise the available sampling equipment.

A number of individual trees were identified within the selected study areas at Summerford and Redding during a site walkover visit.

At Summerford, birch was the predominant species growing on the site and three birch trees where chosen on the basis of their respective locations and size (4 to 5 meters tall). Tree size was important as the selected trees had to be large enough to withstand the rigorous sampling program without suffering ill effects or dying in the middle of the experiment.

At Redding, two birch, two willow and one sycamore tree were identified in study area 217 and one birch and one willow were selected in study area H4. Reference should be made to Chapter 3 for details of study sites.

Representative biomass samples were collected at monthly intervals from each tree between the period from 29 March 1994 through to 1 March 1996. No samples were collected in the period between November 1994 and March 1995 and a few other isolated months when time constraints prevented site visits.

The samples collected from each tree comprised root, core and branch samples. At Summerford 'low' and 'high' level branch samples were collected while at Redding only 'high' level branches were sampled.

High level pruners were utilised in the collection of branch samples located at heights of between 3 and 6 metres above ground level. The pruners comprised four 1 metre sections which could be assembled in increments of 1 metre to adapt to the required

sampling height. At Summerford low level branches were collected at heights below 2 metres utilising hand held pruners.

Core samples were recovered from the tree stem at 0.50 metres above ground level using an incremental corer. The corer comprised a 'Pressler borer' which, when screwed into the tree allowed the collection of a core sample approximately 5 mm in diameter and up to 35 cm in length.

Root samples were exposed by hand digging and care was taken to identify the root leaving the main root bowl of the chosen tree. The root was followed outwards from the main root bowl and extreme care was taken to maximise the recovery of the fine roots.

The sampled vegetation was collected in polythene bags and labelled with permanent marker indicating the date, sample position, site and tree species. After collection the samples were transported to the laboratory within a few hours of collection. The samples were stored under refrigerated conditions (3-4 ^{0}C) prior to processing.

2.2.3 Laboratory Preparation of 'Field' Samples

As discussed by Hoffmann & Lieser (1987), seasonal variations can cause fluctuations in the moisture content of biomass samples. Prior to analysis all samples were oven dried to standard weight. The fresh and dry weights of each sample were recorded to allow the reporting of metal concentrations on either fresh or dry weight basis, however in most instances metal concentrations were reported on oven dry basis.

Core samples were divided into three equal lengths and oven dried to constant weight. No attempt was made to grind the core sample as due to its small quantity of material it was considered a proportion of the sample would be lost in the grinder and recovery of material would be poor.

Root samples were the most susceptible to external contamination from surface adhered soil. In this regard all root samples were subject to a defined washing process. The washing procedure had to rigorous enough to remove all surface soil but gentle enough to prevent leaching of internal metal from the root. Loosely adhered soil was rinsed off under running cold tap water and was aided by very gentle rubbing. The rinsed roots were placed in a large glass beaker and immersed in deionised water and subjected to 15 minutes in an ultrasonic bath. Finally the roots were rinsed in deionised water and blotted dry. The roots were separated visually into fine roots and coarse roots and oven dried to constant weight in an oven at 80 ^{0}C.

Leaves were removed by hand from branch samples and bulked together. The foliage was separated into the individual components of leaves and stemwood. As discussed previously when undertaking plant uptake studies it is important to consider atmospheric contamination of foliage and where atmospheric contamination is expected the foliage should be washed to remove the surface contamination. However, given that both study sites were within a few miles of each other and remote from major roads, the aerial biomass samples were not washed.

The stemwood was further divided into the components of fine twigs, wood and bark. All tertiary and some of the finer secondary branches were chopped up into short lengths and labelled as fine twigs. The remaining primary and larger secondary branch samples were cut into 3 to 5 cm lengths and the bark removed to give both wood and bark components. The wood samples were further cut into short lengths to aid grinding. The segregated samples of leaves, fine twigs, wood and bark were oven dried at 80 ^{0}C for a period of 4 days before being removed and cooled in a desiccator prior to grinding.

The samples were ground in a rotary cross-beater mill with a stainless steel grinding chamber which was fitted with a 1.5 mm stainless steel sieve. Laboratory prepared samples were retained in labelled polythene bags awaiting chemical analysis.

2.3 LABORATORY ANALYSIS OF SOIL

2.3.1 Soil pH

Introduction

Soil pH is a measure of the hydrogen ion concentration within the soil. Soil pH is an important determination as the pH of a soil can influence the solubility and availability of metals in soils.

Soil pH values can be determined by shaking a known weight of soil with a known volume of solution and measuring the soil pH in the resulting soil/solution suspension using a pH meter and probe. It is important to understand that the analysis of soil pH can be influenced by a number of factors including the pre-drying of the soil, carbon dioxide content, soluble salt content and the soil : solution ratio used.

The procedure adopted was a 1:2.5 (soil : solution) ratio, where the choice of solution was either deionised water or 0.01M calcium chloride (MAFF, 1986).

Procedure

Soil pH was measured in duplicate for each sample. A 10g sub-sample of air dried and sieved soil as prepared in accordance with the documented procedure (Section 2.1.5) was weighed into a clean glass bottle.

Depending on the method chosen a 25 ml aliquot of deionised water or calcium chloride was dispensed into the bottle. The bottle lid was replaced and the soil solution shaken intermittently for 30 minutes. After 30 minutes the soil-water suspension was agitated and the pH electrode lowered into the suspension. A 30 second stabilisation period was allowed for before recording the pH value of the soil. The pH electrode was carefully rinsed with deionised water to remove traces of soil between samples. The pH meter was calibrated prior to use with prepared buffer

solutions (pH 4 and 7). A soil pH value of 7 is regarded as neutral and neither acidic or alkali. Due to variations between soils slight acidic or alkali soils are considered normal. In this regard a soil is considered to have an unusual pH when the value falls out with the range 5.5 to 9.5 (ICRCL 89/83).

2.3.2 Soil Salinity

Introduction

The ease with which a plant can take up water from the soil depends not only on the presence of soil water content also on the concentration of dissolved salts in the soil solution. Water uptake is driven by the osmotic gradient created within plant cells which contain higher concentrations of dissolved salts. As the osmotic potential of the soil solution increases with increasing levels of dissolved salt there comes a point where plants begin to experience difficulties in taking up water. This situation arises most often in irrigated crops where irrigation water containing high concentrations of dissolved salts increases the salinity of the soil. Soil salinity may also be a factor in waste spoils where contamination levels are high and dissolved heavy metal salts increase the salinity.

The electrical conductivity of aqueous solutions is directly related to the concentration of ions in solution. Very pure water has a low conductivity while concentrated solutions of electrolytes have high conductivity values. Conductivity measurements of soil solution and soil extracts are a direct measure of the concentration of ions in solution. Conductivity is the reciprocal of electrical resistance and is usually expressed in siemen cm^{-1} or mho cm^{-1}.

Procedure

Soil salinity was assessed by measuring the conductivity of soil water extracts. Duplicate 20g samples of air dry, sieved soil, prepared in accordance with the

documented method (Section 2.1.4) were weighed into separate screw cap glass bottles. A 50 ml aliquot of deionised water was dispensed into each bottle and the bottle lids replaced tightly. The bottles were shaken intermittently for 15 minutes and the solutions filtered through Whatman No. 1 filter papers.

A calibrated conductivity probe zeroed in deionised water was immersed in the filtered solutions and the conductivity readings recorded in either μSiemens cm^{-1} or mSiemens cm^{-1} depending on the relative conductivity of the soil extracts. The conductivity probe was rinsed thoroughly with deionised water between samples.

2.3.3 Loss on Ignition

Introduction

Organic matter is a fundamental component of soil which can influence the behaviour and classification of soils. Wild (1988) suggests that arable topsoil can contain between 1 and 3 % organic carbon, however, grassland and forest soils can contain higher levels especially when soils are waterlogged or acidic as such conditions can reduce microbial breakdown of organic matter which leads to an accumulation of soil organic matter. Determination of the organic matter content of a soil can be obtained by destroying the organic matter and recording the resulting loss in weight. One procedure which has been developed for determining the organic matter content of a soil is the gravimetric assessment of organic matter destroyed by high temperature destruction, termed 'loss on ignition' (MAFF, 1986).

A soil heated in a furnace to 700 ^{0}C will lose weight due to the ignition of organic matter and the loss of strongly held hygroscopic and combined water and carbonate in calcareous soils. However, the losses of combined water and carbonate can be reduced by lowering the furnace temperature.

Procedure

Duplicate, clean, pre-weighed vitrosil basins containing known weights of oven dried soil (approximately 10g) were placed in a muffle furnace. It was important to note the order of basins within the furnace as identification labels are burned from the basins by the high furnace temperature. The furnace was switched on and the samples were heated to 500 ^{0}C and held at that temperature for 6 hours.

After a period of cooling the basins were transferred to a desiccator and cooled to room temperature prior to re-weighing. The recorded weights were used to calculate percentage loss on ignition (% LOI) using the following equation:

Calculation

$$\%LOI = \frac{\text{wt.oven dry soil - wt. ignited soil}}{\text{wt. oven dry soil}} \times \frac{100}{1}$$

2.3.4 Particle Size Fractionation

Introduction

Soil texture is a description of the size distribution of particles in a soil (clay, silt and sand). The textural class of a soil can be determined from its particle size distribution whereby the soil classes fit into commonly used 'farming' descriptions such as clayey, loamy or sandy. The type of classification was developed by Childs (1974) with the particle sizes defined as sand (2 to 0.05 mm), silt (0.05 to 0.002 mm) and clay (< 0.002 mm). In order to measure the particle size distribution the soil is broken down into its primary particles. Different sized components of the soil are then separated by sieving and sedimentation determinations. The procedure developed by MAFF (1986) uses both chemical and physical processes for separating the primary soil components prior to sieving.

Procedure

A 10g sub-sample of air dry sieved soil prepared in accordance with documented procedures (Section 2.1.5) was weighed accurately into a clean 400 ml beaker. A 50 ml aliquot of 6% hydrogen peroxide and a few drops of anti foaming agent were dispensed in to the beaker. After the initial reaction had subsided the beaker and contents were heated on a steam bath for approximately 30 minutes until the reaction ceased. The solution was allowed to cool before a further 50 ml aliquot of hydrogen peroxide was added and the solution heated on a steam bath. This staged approach was continued until the addition of hydrogen peroxide caused no further reaction or effervesce. The sides of the beaker were washed down with distilled water and 10 ml

of a 5.7% Calgon solution added. The suspension was subjected to five minutes in an ultrasonic bath.

The fine and medium to coarse sand particles were separated by sieving through 53μm and 180μm sieves respectively. The soil was carefully rinsed from the beaker into the 180μm sieve which was fitted above the 53μm sieve. The soil solution was washed through both sieves and the resulting filtrate containing the clay and silt fractions collected in a 1000 ml graduated cylinder.

The soil fraction retained on the 180μm sieve was transferred into a pre-weighed clean ceramic evaporating basin and labelled medium to coarse sand. The fine sand fraction recovered from the 53μm sieve was transferred into a similar evaporating basin which was labelled fine sand. Excess water was evaporated off prior to drying the samples in the oven at 100 °C.

The method of analysing the finer particles of silt and clay was based on the sedimentation rates of the particles. The relationship between the radius of a particle and its settling velocity in a medium of given viscosity is stated by 'Stokes' Law.

$$V = \frac{g(\sigma - \rho)d^2}{18\eta}$$

Where V = settling velocity cm s^{-1}

g = acceleration due to gravity (981 cm s^{-1})

σ = assumed density of settling particle (2.6 g cm^{-3})

ρ = density of water (0.998 g cm^{-3} at 20 ^{0}C)

η = viscosity of water (0.010 g s^{-1} cm^{-1} at 20 ^{0}C)

d = diameter of particle (equivalent settling diameter, cm)

The 1000 ml graduated cylinder was made to volume with distilled water and the temperature of the solution recorded. Using 'Stokes' Law, a sampling depth and corresponding sampling time was calculated.

The graduated cylinder was stoppered with a rubber bung and the cylinder vigorously shaken to bring all the soil in to suspension. The cylinder was placed on a level surface and a 50 ml volume of the suspension was removed at the calculated depth and time. The sampled suspension was transferred to a basin labelled silt and clay. Duplicate samples of silt and clay were recovered by this procedure. The excess water was evaporated off and the samples dried in an oven at 100 °C.

To recover clay samples the graduated cylinder was shaken and placed in a water bath maintained at a constant temperature of 25 ^{0}C for a period of approximately 8 hours. Care was taken not to agitate the graduated cylinder and 25 ml aliquots of the soil suspension were removed at the correct time for the calculated depth using 'Stokes' Law. The sampling was undertaken in duplicate and the excess water evaporated and the samples oven dried to constant weight.

The oven dry samples were removed from the oven and cooled in a desiccator prior to re-weighing. The recorded weights of the empty basins and basins with samples were used to calculate the particle size determination (PSD) using the following equations:

Calculations

(1) $$\textit{Wt. oven dry mineral soil} = \textit{Wt. Soil} \times \textit{Conversion Factor} \times \frac{(100 - \%\ LOI)}{100}$$

(2) $$\%\ \textit{Sand Fraction} = \frac{\textit{Weight of sand fraction}}{\textit{Weight of oven dry mineral soil}} \times \frac{100}{1}$$

(3) $$\%\ \textit{Silt + Clay} = \frac{\textit{Wt. Fraction - Wt. Dispersant}}{\textit{Wt. oven dry mineral Soil}} \times \frac{\textit{Vol. cylinder}}{\textit{vol. pipette}} \times \frac{100}{1}$$

(4) $$\textit{Wt. Dispersant(g)} = \frac{0.57 \times \textit{volume pipette}}{1000}$$

$$(5) \quad \%\ Clay = \frac{wt.\ fraction - wt\ dispersant}{Wt\ oven\ dry\ mineral\ soil} \times \frac{vol.\ cylinder}{vol.\ pipette} \times \frac{100}{1}$$

$$(6) \quad \%\ Silt = \%\ Silt + Clay - \%\ Clay$$

The procedure is widely used to determine the percentage of clay, sand and organic matter content of a soil and can be used to define textural classes of soils. Soil classification has important implications on the fertility of soils in agriculture, nutrient status, ability to hold cations and the construction engineering.

2.3.5 Cation Exchange Capacity

Introduction

This measurement of the cation exchange capacity gives the total quantity of negative charge in a soil with the capacity to hold exchangeable cations. In simplistic terms this can be thought of as the soils nutrient holding power. In a soil the majority of the cation exchange capacity is made up of fixed charges on the surface of clay minerals and pH dependent charges on clay edges and carboxylic and phenolic groups on soil organic matter. It is therefore important to state at what pH the cation exchange capacity was measured. The method used was an adaptation of the Schofield (1949) method which involves the saturation of all the negative charge sites with K^+ ions at pH 7. Excess K^+ ions are removed by washing the soil with 90% ethanol. The K^+ ions held on the negative charge sites are then displaced by leaching the soil with NH_4^+ ions. The measured concentration of K^+ ions displaced can be used to calculate the cation exchange capacity of the soil.

Procedure

1 litre of 1M ammonium acetate was prepared by dissolving 77.06g of the salt in 900 ml of deionised water. The pH was adjusted to 7 by either the addition of acetic acid or ammonia depending on the starting pH. The solution was then made to 1 litre with deionised water.

The 1 litre of 1M potassium acetate was prepared by dissolving 98.14g of salt in 900 ml of deionised water. The pH was adjusted to 7 by either the addition of acetic acid or potassium hydroxide and made to volume with deionised water.

Triplicate or duplicate samples of approximately 10g of air dry, sieved soil were weighed out accurately. This soil was mixed thoroughly with an equal volume of acid washed sand to assist the percolation of the leaching and washing solutions. A glass leaching column was plugged with glass wool, leaving a level platform. The soil and sand mix was poured in to the leaching column and another plug placed on top. The top plug was required to prevent disturbance of the top layer of soil.

The soil columns were leached with 200 ml of 1M potassium acetate at pH 7 and the leachates discarded. The columns were then leached with 100 ml of 90% ethanol and the leachate discarded. The soils were further leached with 200 ml of 1N ammonium acetate at pH 7. The leachates were collected in 250 ml volumetric flasks and made to volume with ammonium acetate. Using prepared standards of 0 to 10 mg l^{-1} of potassium in 1M ammonium acetate the concentration of the potassium in the solutions was measured by flame photometer. For leachate samples containing potassium concentrations in excess of the stand graph calibration range the solutions were diluted by a recorded dilution factor.

From the analysed potassium concentration the cation exchange capacity (CEC) of each soil was calculated using the following equations:

1. *weight of oven dry soil* = *wt. air dry soil x conversion factor (C.F.)*

2. K^+ *held on soil (mg* kg^{-1}*)* = $\frac{\text{solution } K^+ \text{ conc}^n \text{ x dilution factor x 250}}{\text{wt. oven dry soil}}$

3. *CEC (me/100g)* = $\frac{\text{Soil } K^+ \text{ (mg/100g)}}{\text{Equivalent wt. K}}$

4. *Equivalent Weight* = $\frac{\text{Atomic weight}}{\text{Valence}}$

Note: (1 meq/100g soil = 1 $cmol_c\ kg^{-1}$)

2.3.6 Exchangeable Bases

Introduction

The cation exchange capacity of a soil is a measure of the total negative charge at a set pH which can hold cations within a soil and is a measure of potential soil fertility. The determination of exchangeable bases is often considered a measure of actual soil fertility.

The major exchangeable cations (exchangeable bases) in British soils are calcium, magnesium, sodium and potassium. In an intensively leached acid soil the exchangeable bases are lost from the soil and as a result the concentrations are low, while in a fertile soil the concentration of exchangeable bases is high. The determination of exchangeable bases is a measure of the concentrations of exchangeable Ca, Mg, Na and K in a soil (MAFF, 1986). When the concentrations of these ions are expressed in milliequivalents per 100g, then the sum of the four values can be expressed as a percentage of the cation exchange capacity, termed base saturation. Well managed arable soils will tend to have higher percentage base saturation than upland or forest soils.

Procedure

Triplicate or duplicate samples of about 10g of the sieved, air dry potting soil were weighed out accurately into small clean beakers and the weight noted. The soils were mixed with equal volumes of acid washed sand and the mixture poured in to leaching columns similar to those used in the cation exchange capacity experiment. The three columns were leached with 200 ml of 1M ammonium acetate at pH 7 and the leachate was collected in 250 ml volumetric flasks. When all the ammonium acetate had leached through the columns the flasks were made to volume using 1M ammonium acetate. The solutions were stored in polythene bottles for analysis to reduce the potential for ammonium acetate leaching potassium from glass.

The concentrations of sodium and potassium were measured by flame emission flame photometry. The standard graph for potassium was 0 to 10 mg ml^{-1} and the standard graph for sodium was 1 to 5 mg ml^{-1}. All standards were prepared in 1M ammonium acetate.

The calcium and magnesium concentration were measured by a atomic absorption spectrophotometer. Strontium chloride was added to the solutions during dilution to overcome interferences in the analytical method. The standard graph for magnesium was 0 to 0.5 mg ml^{-1} and the standard graph for calcium was 0 to 5 mg ml^{-1}.

The cation exchange capacity of each element was calculated in the soil in me/100g soil and the % base saturation calculated using the equation given below:

Calculations

Cation Exchange Capacity calculated individually for each element as in section 2.3.5

$$\%\ base\ saturation = \frac{sum\ of\ Ca, Mg, Na\ \&\ K\ (me/100g) * 100}{Cation\ exchange\ Capacity}$$

2.3.7 Metal Adsorption

Introduction

When metal ions are added to a soil the metal ions are removed from the soil solution and become chelated and fixed within the soil and held on cation exchange sites. As more metal is added these adsorption sites become saturated and less metal is removed from solution. Eventually all the adsorption sites become saturated and no more metal is adsorbed. The only way to predict the exact adsorption of metal salts in an unknown soil is by experimental analysis. As a preliminary indication of how each

metal salt (Cu, Cd, Cr, Ni, Pb and Zn) would behave when added to the soil used in the pot experiments some simple metal adsorption studies were undertaken. The experimental procedures used were those developed by Khattack (1987) during the course of a PhD research project at the University of Glasgow.

Metal adsorption onto soil was measured by shaking a fixed weight of soil with increasing amounts of metal in solution. By analysing the concentration of metal remaining in the equilibrium solution it is possible to calculate the quantity of metal adsorbed by the soil. Soils which adsorb high concentrations of metals are likely to reduce the potential effects of toxic metals to vegetation. In addition metals which are preferentially adsorbed are likely to less toxic when compared with metals adsorbed to a lesser degree.

Procedure

Initially a 1000 mg l^{-1} stock solution of each metal salt (Cu, Cd, Cr, Ni, Pb and Zn) was prepared by dissolving the calculated weight of metal salt in 900 ml of deionised water and making to volume. A range of standards were prepared from these stock solutions (0, 1, 2, 5, 10, 20, 30, 40, 50, 70, 90 & 100 mg l^{-1}). The range had to be expanded up to 400 mg l^{-1} in increments of 50 mg l^{-1} for lead.

1g of the 'pot soil' was accurately weighed into a clean dry glass bottle. This was replicated for each metal concentration and each metal. A 50 ml volume of each standard solution was pipetted into individual bottles and the lids to the bottles were replaced. The bottles were placed on an end-over-end shaker and shaken intermittently for 48 hours. The bottles were removed from the shaker and the solutions filtered through Whatman No. 1 filter papers and the solutions were collected in clean bottles. The filtrates were analysed by flame Atomic Absorption Spectrometry for each respective metal. In addition the metal concentration in the prepared 1 mg l^{-1} standard solutions for each metal were analysed and the value used to calculate a conversion factor to calculate the exact concentration of each stock

solution. The concentration of metal adsorbed by a given soil was calculated using the following calculations:

Calculations

1. *Concentration Conversion Factor* = $\frac{\textit{Measured Conc}^{n} \textit{ of 1 mg } l^{-1} \textit{ std.}}{1}$

2. *Total wt. of metal in Original Solution* = $\frac{\textit{Measured Conc}^{n} \textit{ x 50 ml}}{\textit{1000 ml}}$

3. *Remaining wt. of metal in Solution* = $\frac{\textit{Measured Conc}^{n} \textit{ x 50 ml}}{1000}$

4. *wt. of metal adsorbed by 1g Soil* = $\frac{\textit{Total solution metal - Remaining solution Metal}}{\textit{weight oven dry soil}}$

5. *'Real Concentration'* = *Intended Conc*^{n} *x Conversion Factor*

2.3.8 Soil Pore Water

Introduction

When soluble metal salts are added to a soil it is assumed that initially all the metal is soluble and available for plant uptake. However, after a short period of time metal/soil reactions including precipitation, cation exchange, specific adsorption and chemisorption remove soluble metal from soil solution. The concentrations of metal in the soil solution will decrease with time as more metal is held on the mineral and organic fractions in the soil.

This experiment was designed to measure the initial (24 hours) metal concentration in the soil solution following the addition of a given concentration of soluble metal salt to a soil at 50% moisture. The procedure was developed to be more comparable with the processes occurring in the 'pot soil' experiments. The procedure also would allow the comparison between selective extraction procedures.

Procedure

The method was developed during the course of this research and was adapted from a procedure which was developed to extract soil solution (Merian *et al.*, 1980). A 20g sub-sample of the air dried, 2 mm sieved pot soil was weighed out into clean polythene centrifuge tubes and the accurate weight of soil noted. The respective weight of metal salt required to create the same soil metal concentrations as used in the Pot experiment was calculated with respect to 20g of soil (Refer calculations 1 and 2). The calculated weights of respective metal salts were accurately weighed into the centrifuge tubes containing the soil. The soil and salt were mixed thoroughly and 10 ml of deionised water was pipetted into each tube. The lids were replaced and the tubes left to stand for 24 hours. After 24 hours the tubes were placed in a centrifuge and centrifuged at 2500 rpm for fifteen minutes. They were removed carefully to prevent disturbance of the solution which collected at the top of the soil layer. A 1 ml aliquot of each solution was pipetted off into a clean 100 ml volumetric flask and made to volume using deionised water. The metal concentrations in the respective solutions were determined by atomic adsorption spectrometry.

Calculations

1. $$\textit{wt. salt require for 1g of metal} = \frac{\textit{Formula wt. salt}}{\textit{Atomic wt. metal}}$$

2. $$\textit{Wt. salt required in 20g soil} = \frac{\textit{Soil conc}^{n}\textit{(g kg}^{-1}\textit{)} \times \textit{20g} \times \textit{wt. salt for 1g metal}}{\textit{1000g}}$$

2.3.9 Acid Digestion

Introduction

When assessing whether a soil is contaminated by heavy metals it is important to measure the total metal concentration within that soil. However, the majority of analytical determinations require that the chemical determinand is in solution. The average concentrations of cadmium, copper, chromium, nickel, lead and zinc in rocks have been reported as 0.2, 55, 100, 75, 13 and 70 mg kg^{-1} respectively (Davis, 1980 and Mason and Moore, 1982). Ure and Berrow (1982) give values for the mean concentration of copper, nickel and zinc in soil as 26, 34 and 60 mg kg^{-1} respectively.

The most widespread method for determining the total metal content of selected soils is the complete digestion of the soil with concentrated acid solutions at elevated temperatures. This procedure dissolves and extracts all the metal present in the soil. There are many different acids and acid mixtures which have been developed for the digestion of soil. Preference has been given to the 'aqua-regia' extraction procedure which comprises a mixture of hydrochloric and nitric acid (MAFF, 1986). The aqua-regia procedure has been further developed in house and weights of soil and acid volumes have been amended to reflect available laboratory equipment.

Procedure

Approximately 0.5g of the air dried and ground soil was weighed accurately onto a small aluminium 'boat'. The weight of the soil was noted and the soil tipped into a clean dry glass boiling tube. The boat was re-weighed and the weight noted. This process was carried out in duplicate and/or triplicate for each soil sample.

A 7.5 ml aliquot of 6M hydrochloric acid was added to each tube and the soil/acid suspensions left for a period of a few hours. The 6M hydrochloric acid was prepared from concentrated 'Analar' grade hydrochloric acid. Subsequently a 2.5 ml aliquot of concentrated nitric acid was dispensed into each tube. The digestion block comprised

40 glass boiling tubes which were slotted into an aluminium heating block. A vacuum extraction unit was fitted above the tubes to remove acid fumes during digestion.

The power to the heating block was turned on and the temperature raised to 120 ^{0}C for a period of 2 hours, after 2 hours the temperature was increased to 140 ^{0}C for the final hour of digestion. After cooling the digests were filtered through Whatman No. 50 filter papers into 50 ml volumetric flasks, the boiling tubes were rinsed 3 times with a small volume of deionised water and the filtrate collected in clean 50 ml volumetric flasks. After filtering the flasks were made to volume with deionised water.

The samples were analysed by flame atomic absorption.

Calculation

$$\textit{Soil Metal Conc}^{n} = \frac{\textit{Measured Conc}^{n}\ \textit{(AAS) x Volume (l)}}{\textit{Weight of Soil}\ \textbf{(kg)}}$$

2.3.10 Selective Extractants for Soil

Background

The total elemental content of a soil is an important factor in determining whether a soil contains elevated concentrations of heavy metals but is often of little significance to a plant as it is generally only available elements which can be taken up by plant roots. In most instances it is only a small percentage of the total metal concentration in the soil that is available for plant uptake.

Selective soil extraction is a procedure which has been developed to remove specific fractions of metal from a soil. A single extractant removes a specific pool of metal from a soil and as a result various extractants can be used in order of increasing strength to assess the concentration of elements present in different pools. From such studies it is possible to predict the availability and likely toxicity of metals in a soil and obtain indices of plant-available micronutrients.

Selective extractants tend to be dilute solutions of mineral or organic acid, simple salts or organic and inorganic complexing agents which are used in a pre-determined order of increasing vigour of attack to remove different pools of metals from the soil.

There are two variations to the use of selective extractants, termed, either sequential or non-sequentially extraction. A sequential extraction requires subjecting the same fraction of soil, say 5g, to a sequence of different extractants. The order of extractants used has to be from weak to strong. Non-sequential extraction is undertaken on sub-samples of a uniform soil and only uses one extractant on each sub-sample. The preferred option used during the course of the experimental work was the non-sequential extraction of soil.

The selective extractants used to determine individual pools of metal in study soils in the order of increasing vigour were calcium chloride, ammonium EDTA and acid oxalate. The experimental procedures were developed by Mallik (1993).

2.3.10.1 Calcium Chloride (0.05M and 0.5M)

Preparation

0.05M and 0.5M solutions of calcium chloride ($CaCl_2$) were prepared by dissolving 11.099g and 110.99g of calcium chloride salt in 1800 ml of 'purite' deionised water, respectively, and making to volume with deionised water in a 2000 ml volumetric flask.

Procedure

Duplicate or triplicate 5g sub-samples of air dried, sieved soil were accurately weighed into clean glass bottles. A 50 ml aliquot 0.05M or 0.5M calcium chloride was dispensed into each bottle from an automatic dispenser. The bottles were placed on a end-over-end shaker and shaken for 16 hours at room temperature. The resulting suspensions were filtered through Whatman No. 1 filter papers, and the filtrates collected in clean 60 ml polythene bottles. The Atomic Absorption calibration standards were prepared in the respective calcium chloride solutions and the sample filtrates analysed by AAS.

The calcium chloride extractant was used to remove heavy metals from the soil solution, exchangeable and weakly fixed fractions in the soil. The concentration of metal extracted by calcium chloride was calculated using the following equation:

Calculation

$$\textit{Conc}^{n}\ \textit{of metal extracted by } CaCl_2 = \frac{\textit{Measured Conc}^{n} \times \textit{Volume of solution}}{\textit{Weight of Soil}}$$

2.3.10.2 0.05M Ammonium EDTA (NH_4 EDTA) at pH 7

Preparation

Two litres of 0.05M Ammonium EDTA was prepared by dissolving 29.20g of ethylenediaminetetraacetic acid salt in 1900 ml of deionised water containing 16 ml of the Analar ammonia solution. The pH of the solution was checked and adjusted to 7 by the addition of either a few drops of dilute hydrochloric acid or ammonia. The solution volume was made to volume with deionised water.

Procedure

Duplicate or triplicate 1g sub-samples of air dry sieved soil were accurately weighed in to clean glass bottles. A 50 ml aliquot of 0.05M EDTA was dispensed into each bottle and the lids replaced. The bottles were shaken on an end-over-end shaker for 16 hours at room temperature. The suspensions were filtered through Whatman No. 1 filter papers and the filtrates were collected in clean 60 ml polythene bottles.

The atomic adsorption calibration standards were prepared in 0.05M EDTA to limit matrix interferences during analysis and the filtrates were analysed by flame atomic adsorption. Ammonium EDTA was used to extract the strongly fixed and organically bound fractions of metal from the soil. The concentrations of metal extracted by ammonium EDTA were calculated using the following equation:

Calculation

$$\textit{Conc}^{n}\ \textit{of metal extracted by } NH_4\ \textit{EDTA} = \frac{\textit{Measured Conc}^{n} \times \textit{Volume of solution}}{\textit{Weight of Soil}}$$

2.3.10.3 Acid Oxalate (0.1M Oxalic Acid/0.175M NH_4 Oxalate)

Preparation

Two litres of acid oxalate solution were prepared by dissolving 25.214g of oxalic acid and 49.74g of ammonium oxalate in 1800-1900 ml of deionised water. The solution was made to volume in a volumetric flask with deionised water.

Procedure

Duplicate or triplicate 1g sub-samples of the air dry sieved soil were accurately weighed into glass bottles. A 50 ml aliquot of the prepared 0.1M acid oxalate was dispensed into each bottle and the lids replaced. The solutions were shaken on an end-over-end shaker for 16 hours.

The suspensions were filtered through Whatman No. 1 filter papers and the filtrate collected in clean 60 ml polythene bottles. Atomic absorption calibration standards were prepared in 0.1M acid oxalate solution to limit matrix interferences during analysis and the filtrates were analysed by flame atomic absorption.

Acid oxalate was used to extract oxide bound soil metal and the concentration of metal extracted by acid oxalate was calculated using the following equation:

Calculation

$$\textit{Conc}^{n}\ \textit{of metal extracted by Acid Oxalate} = \frac{\textit{Measured Conc}^{n}\ \textit{x Volume of solution}}{\textit{Weight of Soil}}$$

It is important to note that when selective extractants are used non-sequentially it is important to subtract metal concentrations obtained for different samples to obtain the

concentrations of metals residing in different pools within the soil. In this regard the following calculations apply:

Calculations

1. *Exchangeable fraction* = *Conc^n of metal extracted by $CaCl_2$*

2. *Strongly bound and organically bound* = *Conc^n extracted by NH_4 EDTA* _ *Conc^n Extracted by $CaCl_2$*

3. *Oxide bound metal* = *Conc^n extracted by Acid Oxalate* _ *Conc^n extracted by NH_4 EDTA*

2.4 LABORATORY ANALYSIS OF BIOMASS

2.4.1 Acid Digestion

Introduction

As with soil it is necessary when analysing the metal content of biomass to release the metals held within the biomass into solution. Acid digestion oxidises the organic matter in the sample and releases the elements previously bound in the biomass into the acidic solution. The released elements can be analysed in the aqueous digest by atomic absorption. The analytical procedure for analysing plant biomass was developed during the course of this research. The effectiveness of the digestion and reproducibility was validated with the purchase of 'Standard Reference Materials'. Details of this validation is given in Section 2.5.5.

Procedure

Approximately 0.2500g of oven dried and ground plant material (Refer Section 2.2) was weighed out accurately onto a small aluminium weighing boat. The plant material was transferred to a digestion tube and the boat re-weighed. Each sample was analysed in duplicate or triplicate.

A 10 ml aliquot of concentrated Analar nitric acid was dispensed into each tube and the tubes allowed to stand for a period of a 2-3 hours. The heating block was switched on and the digestion run at a temperature of 140 ^{0}C for 3 hours before allowing the solutions to cool. The samples were filtered through Whatman No. 50 filter papers and the filtrates collected in clean 25 ml volumetric flasks. The tubes and filter papers were washed 3-4 times with small aliquots of deionised water which in turn were collected in the volumetric flasks. The flasks were made to volume with deionised water.

The filtrates were analysed for cadmium, copper, chromium, nickel, lead and zinc by flame atomic absorption spectrometry. The concentrations of metal in the biomass was calculated using the following equation:

Calculation

$$\textit{Biomass Metal Conc}^{n} = \frac{\textit{Measured Conc}^{n} \times \textit{Volume of Solution}}{\textit{Weight of biomass}}$$

2.4.2 Selective Extraction Of Biomass

Introduction

As previously discussed the selective extraction of metals from soil is a useful method for differentiating between different pools of metals in the soil.

In plant systems nutrients predominantly enter vegetation via the roots from the soil solution. The method of uptake is complex, however, in simplistic terms nutrients in the soil are taken up from the soil solution by the plant roots. Some metals are believed to bind to the external surface of plant roots while others are transported across root cell membranes and are likely to be held internally within the cell membranes and cell walls. Selective extraction procedures have been developed from selective extraction methods (Mallik, 1993).

Selective extraction techniques have been principally used in an attempt to differentiate between different pools of metals held at and on the surface of plant roots.

The selective extraction of metals from the surface of plant roots was undertaken using two selective extractants followed by acid digestion. The order of sequential extraction was 0.05M ammonium acetate at pH 7, followed by 0.05M ammonium EDTA (NH_4 EDTA) at pH 7 followed by residual elemental analysis.

Procedure

Two Litres of 0.05M ammonium acetate (NH_4 OAc) was prepared by dissolving 7.708g of ammonium acetate salt in 1800 ml of deionised water. The pH of the solution was adjusted to 7, either by the addition of ammonia or acetic acid and made to volume with deionised water. The preparation of 0.05M NH_4 EDTA was by the procedure documented in Section 2.3.10.

Only fresh roots were used for the experiment and the roots were kept intact to limit metal leaching from the cut ends. Roots were easier to recover from the filter papers during the process of sequential extraction.

The fresh roots were initially rinsed in deionised water to remove free metal from the root surface, the roots were given a short flush with deionised water. Duplicate or triplicate sub-samples of approximately 5 g were weighed into separate glass bottles. A 50 ml aliquot of ammonium acetate was dispensed into each bottle and the lids replaced. The bottles were shaken for 2 hours on an end-over-end shaker. After the shaking the extracts were filtered through Whatman No. 1 filter papers and the filtrates collected in clean polythene bottles.

All of the root sub-samples were returned to the respective glass bottles. A 50 ml aliquot of ammonium EDTA was dispensed in to each bottle and the bottles were shaken for 2 hours on an end-over-end shaker and the extracts were filtered through Whatman No. 1 filter papers and the filtrates were collected in clean polythene bottles. The root samples were oven dried at 80 ^{0}C for 3-4 days before grinding in the hammer-cutter-mill. The residual total metal content was measured by complete digestion in concentrated nitric acid.

All metal analysis was undertaken by atomic absorption and calibration standards were prepared in the respective extracting solutions. The concentration of metal extracted by the respective extracting solutions were calculated using the following equations:

Calculations

1. $$\textit{Conc}^{n}\textit{ of Metal extracted Ammonium Acetate} = \frac{\textit{Measured Conc}^{n}\textit{ x Volume of Extractant}}{\textit{Weight of root (fresh)}}$$

2. $$\textit{Conc}^{n}\textit{ of Metal extracted Ammonium EDTA} = \frac{\textit{Measured Conc}^{n}\textit{ x Volume of Extractant}}{\textit{Weight of root (fresh)}}$$

3. ***Residual Metal*** = $\frac{\textit{Measured Conc}^{n} \textit{ x Volume of Extractant}}{\textit{Weight of root (dry Wt)}}$

2.5 INSTRUMENT SET-UP AND OPERATION

2.5.1 Flame Atomic Absorption

Background

The principles of Atomic Absorption Spectrophotometry for the quantitative assessment of metal atoms is discussed in the following Section. Specific instrument set-up parameters and principles of operation refer to the Perkin Elmer 1100B instrument which have been summarised from the Perkin Elmer operations manual and handbook (1982).

When a 'ground state' atom absorbs light energy of a specific wavelength the increase in energy is sufficient to shift the atom from 'ground state to an 'excited state'. As the number of atoms in the light path increases, the amount of light absorbed also increases. By measuring the level of absorption a quantitative determination of the amount of analyte can be made. The use of special light sources and careful selection of wavelengths allows the specific determination of individual elements.

There are 5 basic components to an absorption instrument:

- The light source which emits the light spectrum of the element of interest
- An 'absorption cell' in which atoms of the sample are produced (flame, graphite furnace)
- A monochromator for light dispersion
- A detector which measures the light intensity and amplifies the signal
- A display that shows the reading after it has been processed

Since atoms absorb light at very specific wavelengths it is necessary to use a narrow-line source which emits narrow-line spectra of the element of interest. The hollow cathode lamp is an excellent, bright, stable line source and is suitable for most

elements except for some volatile elements where it is necessary to use a electrode discharge lamp.

The cathode is a hollowed-out cylinder constructed entirely or in part of the metal whose spectrum is to be produced. The anode and cathode are sealed in a glass cylinder with neon or argon. An electrical potential is applied between the anode and cathode and some of the fill gas atoms are ionised. The positively charged ions collide with the negatively charged cathode and dislodge metal atoms in a process called 'spluttering'. Spluttered metal atoms are further excited and emit light through impact with the fill gas. Every label on a hollow cathode lamp has a suggested operating and maximum current for continuous and modulated operation.

Set-up

Instrument set-up will vary with model of atomic adsorption spectrometer, the operational details given are for a Perkin-Elmer 1100B. A 10 cm burner head was used for all determinations made with a air-acetylene flame. This gave a long burner path to provide increased sensitivity. Air-acetylene is the preferred flame for the determination of approximately 35 elements and the temperature of the flame is in the region of 2300 ^{0}C. The acetylene flow can be varied between 2.5 and 3.5 litres/minute depending on the set-up for individual elements. The air flow used was 8.5 litres/minute.

During instrument set-up it was important to optimise sensitivity by adjusting the burner head relative to the light path of the instrument, altering of the fuel/oxidant ratio and adjusting the nebulizer.

Calibration

Quantitative measurements in atomic absorption are based on Beer's Law, which states that concentration is proportional to absorption (C = kA). At higher concentrations the relationship between concentration and absorption deviates from the law and is not linear.

The top end of the linear range for most elements is between 0.25 and 0.30 absorption units. For the elements of Ni and Zn the range was often extended beyond the linear range. In such cases it was important to provide a sufficient number of calibration standards beyond the linear to calibrate the instrument.

Sensitivity versus Detection

Sensitivity in atomic absorption is defined as the concentration of an element (expressed in mg l^{-1}) required to produce a signal of 1% absorption (0.0044 absorption units). The sensitivity of the instrument was determined by noting the absorption value produced by a known concentration of element within the linear range using the following calculations.

1. $$\frac{\text{Conc}^{n}.\ of\ Std.}{Measured\ Abs.} = \frac{Sensitivity}{0.0044}$$

or

2 $$Sensitivity = \frac{\text{Conc}^{n}.\ of\ Std\ x\ 0.0044}{Measured\ Abs.}$$

A sensitivity calculation can determine if the instrument parameters are optimised and the instrument is performing up to specification.

The detection limit is defined as the concentration of the element which will produce a signal to noise ratio of two. The detection limit is based on the signal amplitude and the baseline noise and is the lowest concentration which can be clearly differentiated from zero.

Interference

Atomic absorption is a very specific technique with few interferences. The interferences that do exist fall into six categories and are defined as chemical

interferences, ionisation interferences, matrix interferences, emission interferences, spectral interferences and background adsorption.

- **Chemical Interference** is the most common interference. If the sample being analysed forms a thermally stable compound with the analyte and is not totally decomposed by the energy of the flame then a chemical interference exists. These chemical interferences can normally be overcome or controlled by the use of a higher flame temperature or the addition of a releasing agent to the samples.

- **Ionisation Interference** occurs when the flame temperature has enough energy to remove an electron from the atom, creating an ion. These electronic rearrangements deplete the number of ground state atoms and atomic absorption is reduced. These interferences can be controlled by the addition of easily ionised sacrificial elements to problem solutions.

- **Matrix interference** can cause either the suppression or enhancement of the analyte signal. These interferences occur when the physical characteristics (viscosity, burning characteristics and surface tension) of the sample and standard differ considerably. The best way to compensate is to match the matrix components in the sample and standard as closely as possible.

- **Spectral Interference** occurs when an absorbing wavelength of an element present in the sample but not being determined falls within the bandwidth of the adsorption line of the element of interest. The problem can be overcome by using a smaller light slit or selecting an alternative wavelength.

- **Background absorption** can be caused by light scattering by particles in the flame and molecular absorption of light from the lamp by molecules in the flame. The way to compensate for background absorption is to use a background corrector. With background correction, simultaneous compensation is obtained at the same wavelength used to measure atomic absorption.

During analytical determinations the matrix of sample and standard solutions were matched as closely as possible. There was no matching of the acid digest matrix with the standards but the results indicated there was no significant interference between the acid digests and the standards prepared in deionised water. During the determination of calcium and magnesium, strontium chloride was added to the samples to act as a releasing agent.

2.5.2 Graphite Furnace Atomic Absorption

Introduction

Graphite Furnace Atomic Absorption Spectrometry (GF AAS) offers a technique for the detection of low concentrations of metal elements by Atomic Absorption. Analysis performed by GF AAS requires a longer analysis time and careful selection of step temperatures to ensure that each process is carried out effectively. Temperatures and suitable time periods for each step are listed for individual elemental determinations (Perkin-Elmer, 1982).

GF AAS techniques were experimented with to determine low level metal concentrations in biomass samples but the technique was not used routinely. The fundamentals of graphite furnace analysis are discussed.

Drying Step

A drying step is employed to evaporate the aqueous phase of the sample. The temperature and time is dependant on the solvent or liquid component. A temperature slightly higher than the solvent boiling point should be chosen, usually 110-150 ^{0}C but is dependant on the nature of the sample. It is important that only rapid evaporation occurs and no boiling is detectable by an audible hissing sound.

Thermal Pre-treatment

A 'Thermal Pre-treatment' (charring step) is used to remove any components of the sample matrix which are more volatile than compounds of the element of interest before atomisation. Parameters have to be optimised for each sample. For dilute aqueous samples a minimum pre-treatment time of 10 seconds at 300-500 ^{0}C is recommended. However, care must be taken to ensure that the time is long enough and the temperature is high enough to volatilise as completely as possible any interfering or 'smoke' producing sample matrix without any loss of analyte. The maximum temperature for thermal pre-treatment is determined by the thermal stability of the element under study. A matrix modifier (salt or acid) can be added in excess to guarantee that the analyte element is converted into the compound of highest thermal stability. The pre-treatment time should be long enough to allow the background signal to return to the baseline before atomisation.

Atomisation Temperature and Time

The temperature selected for atomisation should be high enough to guarantee complete volatilisation of the analyte within a few seconds. The lowest temperature should be chosen which gives maximum sensitivity in peak area or peak height. Total atomisation time (ramp and hold) should be chosen sufficiently long to allow the atomisation signal to return to the base line.

Due to the increased level of detection, the linear range for the studied metals analysed by GF ASS were:

Zinc	0.0-6.0 ppb	
Copper	0.0-150 ppb	
Chromium	0.0-75 ppb	(based on 10 µl injection)
Lead	0.0-125 ppb	
Nickel	0.0-350 ppb	
Cadmium	0.0-7.5 ppb	

The calibration standards were prepared from standard stock solutions (1000 ±2 mg l^{-1}) made to volume with 'Purite' deionised water.

The temperature and time steps used were standard documented procedures documented in the instrument manual using pyrolytically coated graphite tubes and nitrogen gas as the purge gas.

2.5.3 Preparation of Calibration Standards

Introduction

All Atomic Absorption standards were prepared from purchased standard solutions. The majority the standards were 'SpectrosoL' (BDH laboratory supplies) or 'Fisons' standard metal solutions. All stock solutions were 1000 ± 2 mg l^{-1} certified solutions. These stock solutions were used to prepare both calibration and check standards.

Cadmium

The linear range for cadmium on the Perkin Elmer 1100B spectrophotometer in normal Acetylene/Air Flame mode was 0 to 2 mg l^{-1}. Four calibration standards were prepared (0.5, 1, 1.5, and 2.0 mg l^{-1}). A 50 mg l^{-1} intermediate solution was prepared, then 1,2,3 and 4 ml of this solution was pipetted into 100 ml volumetric flasks which was made to volume with deionised water to give respective solution concentrations of 0.5, 1, 1.5 and 2 mg l^{-1}.

Copper

The linear range of copper on the Perkin Elmer 1100B spectrophotometer in normal Acetylene/Air Flame mode was 0 to 5 mg l^{-1}. The procedure was to prepare 5 standards (1, 2, 3, 4 and 5 mg l^{-1}). Initially a 100 mg l^{-1} solution was prepared, then 1

ml, 2 ml, 3 ml, 4 ml and 5 ml of this solution was pipetted into five clean 100 ml volumetric flasks and made to volume with deionised water.

Chromium

The linear range for chromium on the Perkin Elmer 1100B spectrophotometer in normal Acetylene/Air Flame mode was 0 to 5 mg l^{-1}. The procedure was to prepare 5 standards (1, 2, 3, 4 and 5 mg l^{-1}). Initially a 100 mg l^{-1} solution was prepared, then 1 ml, 2 ml, 3 ml, 4 ml and 5 ml of the 100 mg l^{-1} solution was pipetted into five clean 100 ml volumetric flasks and made to volume with deionised water.

Lead

The linear range for Lead on the Perkin Elmer 1100B spectrophotometer in normal Acetylene/Air Flame mode was 0 to 20 mg l^{-1}. The procedure was to prepare 4 standards (5, 10, 15 and 20 mg l^{-1}). Initially a 100 mg l^{-1} solution was prepared, then 5 ml, 10 ml, 15 ml and 20 ml were pipetted into four 100 ml volumetric flasks and made to volume in deionised water.

Nickel

The linear range for Nickel on the Perkin Elmer 1100B spectrophotometer in normal Acetylene/Air Flame mode was 0 to 2 mg l^{-1} but a 7 mg l^{-1} standard was required for the sensitivity check (absorption of 0.2 units). Five calibration standards were prepared (1, 2, 3, 5 and 7 mg l^{-1}) by pipetting 1, 2, 3, 5 and 7 ml of an intermediate 100 mg l^{-1} standard solution into five 100 ml volumetric flasks which were made to volume with deionised water.

Zinc

The linear range for zinc on the Perkin Elmer 1100B spectrophotometer in normal Acetylene/Air Flame mode was 0 to 1 mg l^{-1}. Due to the limited linear range the

calibration graph was extended beyond the linear range to 5 mg l^{-1}. Seven calibration standards were prepared (0.5, 1, 1.5, 2, 3, 4 and 5 mg l^{-1}). A 50 mg l^{-1} solution was prepared and 1, 2, 3,4 6,8 and 10 ml of the 50 mg l^{-1} solution was pipetted into seven 100 ml volumetric flasks which were made to volume with deionised water.

2.5.4 Comparison of AAS and GF-AAS

The limit of detection is often defined as the concentration of an element which will produce a signal to noise ratio of two. A theoretical limit of quantification has been calculated for each metal (Table 2.1). This calculation is related to the representative check standard and the analytical procedure utilised. The level of quantification is based on the following assumptions:

- the sensitivity check standard for each metal gave a signal of 0.200 absorbance units (in reality the sensitivity was generally greater)
- The smallest detectable signal was 0.001 absorbance units.
- The detection limit is the concentration giving a signal/ noise ration of 2. Under optimum condition this is twice the minimum detectable signal.
- Biomass analysis digests were made to 25 ml volume and the weight of biomass was 0.2500g (multiplication factor of 100)

Table 2.1. Theoretical Limits of Quantification

Metal	Minimum Signal (expressed as mg l^{-1})	Minimum Signal x 2	Multiplication factor (Analytical)	Limit of Quantification (mg kg^{-1})
Copper	0.025	0.05	100	5
Chromium	0.025	0.05	100	5
Cadmium	0.01	0.02	100	2
Lead	0.1	0.2	100	20
Nickel	0.035	0.07	100	7
Zinc	0.005	0.01	100	1

The limits of quantification given in Table 2.1 are theoretical levels which should be obtained when instrument parameters are optimised and the base line noise is zero. The minimum signal is also the minimum detectable metal concentration in the analytical solution.

In an attempt to compare results obtained by AAS a small selection of biomass samples were analysed for copper and lead by AAS and Graphite Furnace Atomic Absorption (GF-AAS). The results are tabulated in Table 2.2.

Table 2.2. Comparison between AAS and GF-AAS Results

Birch Tree 2 - 4 ft Bark	Copper		Lead	
	AAS	GF AAS	AAS	GF AAS
March	6.5	6.52	3.0	6.57
April	5.1	6.02	12.6	7.38
June	4.3	5.53	9.2	7.56
July	6.9	5.29	5.9	7.06
August	3.9	5.09	3.6	5.78
September	5.6	6.31	8.0	6.64
October	9.4	5.63	6.5	5.26
Theoretical Limit of Quantification	5	0.15	20	0.125

Note: All values are mg kg^{-1}

No conclusions can be drawn from these results which are included for information. Further definition on the accuracy and reproducibility of the analytical procedure will be given in Section 2.5.5 'Certified Reference Material'.

2.5.5 Certified Reference Material

As a procedure to assess the reproducibility of the plant digestion process some Certified Reference Material was purchased from the Laboratory of the Government Chemist (LGC). LGC prepare a large volume of standard reference material using standard laboratory procedures. The prepared material is then issued to a large

number of commercial laboratories for chemical analysis. The results are returned to LGC for statistically analysis to deduce the concentration of determinands within the sample. After assessment the material is available for purchase as certified reference material with defined concentrations of chemical parameters.

The two Certified Reference Materials purchased were; Olive Leaves (*Olea europaea*) BCR No 62 (Community Bureau of Reference) and Pine Needles, Standard Reference Material 1575 (National Bureau of Standards). The materials were selected because they were the materials which most closely matched the biomass samples to be analysed.

The two standards were primarily used for validating then evaluating the reliability of the analytical methods used for the determination of trace elements in biomass samples. During the acid digestion procedures intermittent samples of standard reference material were treated as test materials. The certified composition of the purchased standard reference material is given in Table 2.3.

Table 2.3. The Certified Composition of Two Standard Reference Materials

Element	Olive Leaves values expressed as mg kg^{-1}	Pine Needles values expressed as mg kg^{-1}
Al	450 ±20	545 ±30
Cd	0.10 ±0.02	* <0.5
Cu	46.6 ±1.8	3.0 ±0.3
Hg	0.28 ±0.02	0.15 ±0.05
Mn	57.0 ±2.4	675 ±15
Pb	25.0 ±1.5	10.8 ±0.5
Zn	16.0 ±0.7	
Cr	* 2	2.6 ±0.2
As	* 0.2	0.21 ±0.04
Ni	* 8	* 3.5

Note

Values denoted with * are values which have not been certified and are included for information only. The uncertainties given make allowances for material heterogeneity, method imprecision and an estimate of possible biases of the analytical methods used.

The analytical results of the standard reference samples included in batch runs have been summarised in Table 2.4. The table also has a column with calculated limit of detection for the analytical procedures which have been calculated using the following equation:

$$\textit{Detection Limit} = \frac{\textit{Standard Conc. x 2 Standard Deviations}}{\textit{Mean}}$$

where;

- Standard concentration is the element concentration in the certified reference material
- Standard deviation is that calculated on the analytical tests undertaken
- The mean is the calculated average of the analytical tests undertaken.

Table 2.4. Acid Digest Results of Certified Reference Material

Element	No	Analysed Results		Certified Composition		
	Analysis	Mean	STDEV	Element Conc	STDEV	Limit of Detection
OLIVE						
zinc	36	14.5	3.1	16	0.7	6.8
copper	28	36.8	1.4	46.6	1.8	3.5
chromium	25	0.4	0.8	2.0*		8
lead	26	21.4	2.9	25	1.5	6.8
nickel	15	1.3	3.0	8*		NC
cadmium	14	0	0.5	0.1	0.02	NC
PINE						
zinc	9	57	4.3	NV		NC
copper	9	2.7	0.8	3.0	0.3	1.8
chromium	9	1.3	0.5	2.6	0.2	2.0
lead	7	9.0	1.4	10.8	0.5	3.4
nickel	3	0.3	0.5	3.5*		11.7
cadmium	7	0.4	0.7	<0.5*		1.8

NOTE: All concentrations are mg kg^{-1} dry weight

* Defines element concentrations which are not certified

NC- value not calculated

NV- no value given

From the calculations made the reproducibility of the metal determination in vegetation for zinc, copper, chromium, lead, nickel and cadmium will be in the order

of ± 7, 3, 5, 5, 10 and 2 mg kg^{-1} respectively. In the following Chapters the tabulated test results given will be as stated ± these calculated values. In the absence of standard reference soil it has been assumed that given the similar multiplication factor of 100 (between volume of solution and weight of digested material) in the soil analysis, the reproducibility of results will be similar.

2.5.6 Flame Emission Spectrometry

For the assessment of sodium and potassium, flame emission spectrometry was used. The principles and operation of the instrument are summarised as follows. When sodium, potassium and lithium are burned in a flame the atoms emit light. Flame emission spectrometry measures this emission of light which is specific to the individual elements (MAFF, 1986). The response can be compared with the response of known concentrations of each element and the concentration in the test solution can be determined.

The correct filter was selected for the element to be analysed. The zero standard was aspirated and the response set to zero using the blank control knob. The sodium and potassium standards were prepared from BDH 'SpectrosoL' (1000±2 mg l^{-1}) standard solution and calibration standards were prepared. The linear range was 0 to 10 mg l^{-1} for potassium and 0 to 5 mg l^{-1} for sodium. The highest standard was aspirated and the reading set to 100. This procedure was repeated until the blank response was zero and the top standard was reading 100. The standard solutions were aspirated and the response noted. During operation the top and bottom scale readings of the instrument were frequently checked using the top standard and blank and adjusted when required.

A calibration graph was plotted as concentration against response and was used to obtain solution concentrations of the respective sodium or potassium in test solutions.

Calculation

$$\textit{Element Conc}^{n} = \frac{\textit{Conc}^{n}\ \textit{in solution} \times \textit{Volume of solution}}{\textit{Weight of Soil}}$$

2.6 POT EXPERIMENT

2.6.1 Introduction

The 'pot' experiment was designed to complement the field and hydroponic studies which are discussed in more detail in Chapter 3 and 5. The experiment was devised to allow the study of a select number of tree species growing in artificially contaminated spoil isolated from the environmental variations often associated with studying vegetation growing in the field. The pot soils were contaminated with soluble inorganic metal salts and the tree seedlings were planted into the artificially contaminated spoil. The experiment was set-up in a greenhouse to exert some environmental control over the experiment.

The greatest problem encountered was the selection of appropriate soil metal concentrations. A number of authors had identified metal concentrations in soils in the vicinity of smelters (Little and Martin, 1972, Hawrys, 1984, Hazlett et al., 1983, Hogan and Wotton, 1984 and Tyler, 1984). The maximum levels recorded were 10000 mg kg^{-1} zinc, 9700 mg kg^{-1} copper, 50 mg kg^{-1} cadmium, 12000 mg kg^{-1} nickel and 5000 mg kg^{-1} lead. Analysis of mine spoil by Borgegard and Rydin (1989) found similar levels for zinc, copper, lead and cadmium. Studies by Eltrop et al. (1991) found *Salix* and *Betula* species growing on mine wastes containing 17000 and 29000 mg kg^{-1} lead. These findings suggested that trees could be extremely tolerant to elevated soil metal concentrations, however, it should be noted that soil contamination arising from smelter emissions is likely to be restricted to the upper surface of the soil.

The most closely related study was undertaken by Smilde (1981). Spruce seedlings (*Picea abies*) were planted in sewage sludge amended with metal salts (cadmium, chromium, copper, nickel, lead and zinc). Growth reductions were noted at the following treatment levels of cadmium (300 mg kg^{-1}), and nickel (250, 500 and 700 mg kg^{-1}), while copper and zinc were toxic at levels of 1000-1500 and 3000 mg kg^{-1} respectively. Lead and chromium levels of 1000-3000 mg kg^{-1} did not have any

observed detrimental effect on plant growth. It should be noted that sewage sludge is likely to have a high organic matter content which may adsorb more metal than a mineral soil, however, metal adsorbed by organic matter is likely to be more plant available in the long term. Given the findings of these studies the study metal concentration generally reflected study concentrations used by Smilde (1981). Amendments to the concentrations were made to reflect metal concentrations identified at the field sites.

The following species were sourced from a commercial nursery: Poplar (*Populus trichocarpa* variety Scott Pauley), Willow (*Salix viminalis* variety Mullatin), Grey Alder (*Alnus incana*) and Lodgepole pine (*Pinus contorta*).

The tree species were selected with regard to the nature of the research project and in this regard reference was made to species used as typical coppice woodland species for the reclamation of contaminated sites (Moffat and McNeill, 1994 and Sennerby-Forsse, 1994).

The selected study species comprised:

- Willow and Poplar which are two commercially important high yielding short rotation coppice woodland species. Some hybrid willow clones have been reported to produce up to 18 dry tons of dry matter per hectare per year (Piper, 1994). In this regard one poplar and one willow species was included in the experimental design.

- Alders have been widely used in the UK for the reclamation of contaminated and reclaimed sites due to their ability, in association with micro-organisms in the root, to fix nitrogen which can improve soil fertility (Hook et al., 1987). Therefore alders have been particularly successful in colonising infertile soils.

- Lodgepole pine has been used widely for site reclamation works. Lodgepole pine is coniferous and by definition remains green all year round, replacing its leaves

every 2-3 years and was chosen to allow comparisons to be made between coniferous and deciduous species (Moffat & MacNeill, 1994).

The soil metal concentrations for the experiment were determined with reference to published information reviewed during the literature review and soil metal concentrations measured in soils collected from the Summerford and Redding sites. The selected metal treatment concentrations for all species were 500 and 3000 mg kg^{-1} zinc, 500 and 2000 mg kg^{-1} copper, 500 and 1000 mg kg^{-1} nickel, 300 and 1000 mg kg^{-1} cadmium, 2000 mg kg^{-1} chromium and 2000 mg kg^{-1} lead.

2.6.2 Set-up

The experimental set-up was self designed and utilised locally available materials. Five tons of uncontaminated topsoil obtained from building excavations was mixed with commercially purchased grit to prepare a topsoil/grit mixture suitable for the experiment. The mixture consisted of approximately ten wheelbarrow loads of soil mixed with five 25 kg bags of medium to coarse grit. After mixing the mixture was passed through a rotating drum garden mixer to break down the soil aggregates and further mix the material.

The weight of metal salt required to create the chosen soil metal concentration was calculated on the basis of each pot containing 8 kg of the prepared soil/grit mix . Due to the overall volume of soil used and the relatively dry nature of the soil no attempt was made to adjust the weight of added salt to take account of the moisture content of the soil. Where possible metal chloride salts were used for Cu, Zn, Ni and Cd, however, for Pb and Cr, nitrate and dichromate were selected because of availability and solubility.

Metal salts were chosen on the basis of solubility in water, although, the availability and cost of the salts were also taken into consideration. It was also important that a similar type of salt was used where possible such as metal chlorides The commercial name, manufacturer and chemical formula for each salt used is given below:

- Cadmium Chloride Hemipentahydrate (Aldrich Chemical Company) [$CdCl_2 .2^1/_2 H_2O$]
- Copper (II) Chloride Hydrate (Aldrich) [$CuCl_2 .xH_2O$]
- Nickel (II) Chloride Hexahydrate (Aldrich) [$NiCl_2 .6H_2O$]
- Zinc Chloride (Aldrich) [$ZnCl_2$]
- Lead Nitrate (Riedel-De Haen Ag Seelze-Hennser) [$Pb(NO_3)$]
- Potassium Dichromate (Riedel-De Haen Ag Seelze-Hennser) [$K_2Cr_2O_7$]

The calculations given below were used to calculate the correct weight of metal salt which was required to give the intended metal concentration in the pot soil allowing for a soil weight of 8 kg. The weight of individual metal salts added to give the required soil metal concentrations are included in Table 2.5.

$$\textit{\% metal in salt} = \frac{\textit{Formula Mass of metal}}{\textit{Formula mass of salt}} \times \frac{100}{1}$$

$$\textit{Weight of salt} = \frac{\textit{Intended soil concentration (mg kg}^{-1}\textit{) x weight of soil (g)}}{\textit{\% metal in salt}}$$

Table 2.5. Weight Of Metal Salts Added To 'Pot' Soils

Soil Metal Treatment	Formula weight of Salt	Weight of salt added to 8 kg of soil (g)
500 zinc	136.28	8.32
3000 zinc	136.28	49.92
500 copper	170.48	8.44
2000 copper	170.48	33.76
500 nickel	237.71	16.2
1000 nickel	237.71	32.4
300 cadmium	228.34	4.87
1000 cadmium	228.34	16.24
2000 chromium	294.19	45.12
2000 lead	331.20	25.6

The chosen tree species were purchased from Tilhill Economic Forestry Ltd, Tillyarthie Nursery, Undy, Ellon, Aberdeenshire. Tilhill is a group of commercial nurseries which produce bare root stock for forestry and amenity planting in the UK.

Eight kilograms of the prepared planting soil was weighed into a large plastic bucket and a pre-weighed weight of the desired metal salt was emptied into the bucket and mixed thoroughly with the soil. Individual seedlings were planted into separate polythene pots using batches of prepared contaminated spoil. The glass bottles containing the metal salts were rinsed with deionised water and the washings were added to the soil. It should be noted all mixing buckets and trowels were washed between different metal treatments.

Ten replicates of each tree species and metal concentration were prepared for zinc, copper, nickel and cadmium, and only five replicates of each tree species and metal concentration were prepared for chromium and lead due to the low level of uptake expected with these metals. In addition the experiment contained twenty five control treatments for each tree species. The control treatments contained no added metal salts. The compete experiment comprised 460 pots.

The pots were arranged into five replicated blocks where the position of trees and metal treatments within the block were randomly selected. Each block contained eight rows of trees (duplicate rows of individual tree species). Tree species were numbered one to eight and the numbers were drawn at random to select the order of species in rows. The metal treatments for each species were labelled A to M, as shown below, and letters were drawn to decide the position of treatments within the rows. The labelling was as follows:

A= 500 zinc

B= 3000 zinc

C= 500 copper

D= 2000 copper

E= 500 Nickel

F= 1000 Nickel

G= 300 cadmium

H=1000 cadmium

I= 2000 lead

J= 2000 chromium

K, L, M = control

An example of the randomised block design used is shown in Table 2.6.

Table 2.6 Randomised Block Design

Willow	**H**	**A**	**B**		**I**	**E**	**L**	**F**	**C**	**D**	**M**	**G**
Willow	**D**	**F**	**E**	**G**	**H**	**J**	**M**	**K**	**C**	**B**	**A**	**G**
Pine	**B**	**J**	**A**	**F**	**D**	**H**	**M**	**C**	**K**	**L**	**E**	**G**
Alder	**I**	**K**	**B**		**E**	**L**	**A**	**H**	**C**	**D**	**G**	**F**
Poplar	**L**	**B**	**C**	**K**	**F**	**H**	**J**	**D**	**M**	**A**	**G**	**E**
Alder	**G**	**M**	**D**	**J**	**E**	**L**	**B**	**F**	**A**	**H**	**K**	**C**
Poplar	**B**	**K**	**F**		**A**	**L**	**G**	**H**	**I**	**C**	**E**	**D**
Pine	**C**	**F**	**D**		**I**	**A**	**E**	**L**	**H**	**K**	**B**	**G**

The pots were laid out on the floor of the greenhouse, however, prior to setting down, the pots were placed in water proof bags which were used as saucers to prevent the metal salts leaching from the pots to the underlying soil and to help retain moisture. The pots were watered at least three times a week, however, during the summer months watering was twice daily. Initially, the intention was to add the water to the pots by hand but due to time constraints watering was undertaken by hosepipe and a fine spray. The fine spray generated a humid atmosphere in the greenhouse during very hot weather and helped to lower the temperature.

The number of trees surviving at the end of the first growing season were assessed and those trees deemed to be dead were replaced by fresh seedlings ordered from Tilhill Nurseries. The trees were replanted during the winter of 1994/1995, directly into the contaminated spoil. Tree survival rates were assessed on four occasions (7 July 1994, 11 February 1995, 20 November 1995 and 19 August 1996). The survival rates are discussed in Chapter 4.

2.6.3 Sampling and Analysis

Initial provision had been made to collect foliage samples from the treatments at monthly periods throughout the growing season over a two year period. However due to the poor survival and small size of some seedlings this was impracticable. Foliage samples were collected by hand on three occasions (June 1994, June 1995 and August 1996). The samples were prepared for chemical analysis in accordance with the procedures detailed in Section 2.4.1.

Soil samples were collected in accordance with the procedures documented in Section 2.1.4 during February 1995 and August 1996. The collected soils were prepared in accordance with the procedures documented in Section 2.1.5 and were analysed for a number of determinands including; soil pH, soil salinity and total metal content. In addition the soils were analysed by selective extraction techniques as documented in Section 2.3 to determine the availability of added metal.

At the end of the experiment intact root samples were collected by cutting open the polythene 'transpot' and carefully separating roots from soil. The results of the biomass and soil chemical tests are discussed in detail in Chapter 4.

2.7 HYDROPONIC STUDIES

2.7.1 Introduction

In the absence of soil, nutrient solutions can provide tree seedlings with the correct supplement of macro and trace nutrients for growth. The 'hydroponic' studies were undertaken to complement the field and pot experiment studies which are discussed in Chapters 3 and 4.

Hydroponic studies offer the advantages to the researcher of being able to study the growth of plants without the influence of soil. Heavy metal salts can be dissolved in the nutrient solutions and the effects of the treatments studied. The correct selection of metal salt is important so that the nutrient solution does not react with and precipitate the heavy metal which is to be studied.

During the literature review a number of the reviewed papers reported the results of metal studies with nutrient solutions. These studies comprised various experiments including studies on filter papers impregnated with solutions (Patterson and Olson, 1983), and experiments where solutions were added to a matrix of sand (Smith and Brennan, 1984). However, in most work reviewed the roots of plants were suspended in nutrient solutions (Scherbatskoy et al., 1986, Moral et al., 1994, Sharma & Sharma, 1993, Turner and Dickinson, 1993, Chiu et al., 1995 and Dushenkov.et al., 1995) to monitor the toxic effect and uptake of added metal. In the above literature solution metal concentrations were generally below 20 mg l^{-1} for most metals studied.

The tanks and equipment required to set-up the hydroponic experiment was sourced locally and self designed with reference to Hershey (1994) and the metal solution concentrations were chosen to cover a wide concentration range. The chosen metal concentrations in nutrient solution were 10, 100 and 500 mg l^{-1} . The 500 mg l^{-1} treatment also corresponded with the low metal treatment in the 'pot experiment' of 500 mg kg^{-1}.

2.7.2 Set-up

The nutrient solution used in the experiment was based on Knops 'complete' nutrient solution and was prepared from a selection of inorganic salts dissolved in deionised water and adjusted to pH 6.5. Details of the weights and types of inorganic salts used are given in Table 2.7. The 'complete' nutrient solution contained calcium, nitrate, potassium, magnesium, sulphur, phosphate, iron, boron, manganese, zinc, copper and molybdenum.

One litre of complete nutrient solution was prepared by pippeting the identified volumes of each stock solution into 500 ml of deionised water. The pH of the solution was adjusted to pH 6.5 and the solution made to volume.

It should be noted that full strength nutrient solution was considered too strong for the transplanted tree seedlings, therefore quarter strength solution was used throughout the duration of the experiment.

Table 2.7. Preparation of Knops 'Complete' Nutrient Solution

Nutrient element	Chemical compound	Supplier	weight (g) in stock solution (100 ml)	volume of stock solution in 1 litre to give full strength nutrient solution
Ca + N	$Ca(NO_3)_2$ $4H_2O$	Riedel-De-Haen Ag Seelze-Hannover	82	1 ml
K + N	KNO_3	Riedel-De-Haen Ag Seelze-Hannover	20	2.5 ml
Mg + S	$MgSO_4$ 7H2O	May & Baker Ltd	49	1 ml
K + P	K_2HPO_4	Hopkin & Williams	14	1 ml
Fe	$FeCl_3$ $6H_2O$ + citric acid	BDH Chemicals Ltd + Koch-Light Laboratories Ltd	2.3	
Trace elements				
B	H_3BO_3	Formachem research international Ltd	2.86	0.10 ml
Mn	$MnSO_4$ $4H_2O$	BDH Chemicals Ltd	1.38	
Zn	$ZnSO_4$ $7H_2O$	BDH Chemicals Ltd	0.22	
Cu	$CuSO_4$ $5H_2O$	Fisons	0.08	
Mo	H_2MoO_4	Riedel-De-Haen Ag Seelze-Hannover	0.09	

The experimental design of the water culture experiment made allowance for the replication of nine different species of trees within each treatment. A total of 18 different treatments were devised comprising six different heavy metals at three different concentrations.

The choice of trees was influenced by the species studied in the 'Field' and 'Pot' experiments and the commercial availability of suitable tree seedlings. The final selection of trees comprised five different species of tree including pine, birch, alder, willow and poplar with more than one variety of alder, willow and poplar. Details of the tree seedlings and the respective ages and sizes supplied by Tilhill Nurseries are provided in Table 2.8.

Table 2.8. Selected Trees for Hydroponic Studies

Seedling Type	Seedling Height	Seedling Age
Lodgepole Pine (*Pinus contorta*)	15-30 cm	2+1
Silver Birch (*Betula pendula*)	30-50 cm	1u1
Common Alder (*Alnus glutinosa*)	20-40 cm	1u1
Grey Alder (*Alnus incana*)	20-40 cm	1+1
Red Alder (*Alnus rubra*)	20-40 cm	1u1
Poplar (*Populus trichocarpa*)	100-150 cm	cutting+1
Poplar (*Populus x euroamericana*)	60-90 cm	cutting+1
Willow (*Salix caprea*)	60-90 cm	cutting+1
Willow (*Salix viminalis*)	60-90 cm	0+1

Both poplar (*Populus trichocarpa*) and willow (*Salix viminalis*) seedlings were used in the 'Pot' Experiment discussed in Chapter 4. Willow and poplar are generally regarded as the highest yielding coppice woodland species. Alders were historically planted during site reclamation works including initial reclamation and screening of coal bings. Alders in association with root micro-organisms are nitrogen fixing which gives them a distinct advantage over other tree species when colonising nutrient deficient soils. Silver birch was found to be growing extensively at both 'field' sites and was studied during the 'field' experiment discussed in Chapter 3. Lodgepole pine was the conifer species studied in the 'Pot' Experiment.

All tree seedlings were purchased from Tillhill Nurseries which is a commercial nursery supplying trees for commercial and amenity planting and represents commercially available species at that time.

The apparatus for the experiment comprised 19 large nutrient solution tanks which allowed for the study of six heavy metals, each at three different concentrations and a control. The tanks were plastic window boxes approximately 0.7 x 0.3 x 0.3 metres in size and were capable of holding 16 litres of solution when full. Plywood panels approximately 0.7 x 0.3 metres were inserted into the top of the tanks to support the tree seedlings. 2.5 cm diameter holes were drilled in the wood panels and the seedlings were inserted through the holes into the nutrient solutions. The seedlings were supported in an upright position by small collars of foam between the seedling stem and the wood panel.

Small air stones, like the type used in aquariums were attached to air pumps and were inserted into the nutrient solutions. The nutrient solutions were aerated throughout the duration of the experiment for 30 minutes on a daily basis.

The six metals selected for the study were copper, zinc, cadmium, nickel, chromium and lead. The same metal salts used in the 'pot' experiment were used and consideration was also given to the reaction of the metal salts with the nutrient solutions and in this regard phosphate was omitted from the lead treatment to prevent precipitation of the lead phosphate.

Details of the metal salts used including the weight of each salt added to the 16 litres of nutrient solution are given in Table 2.9. The weights of salt were calculated using the following equation:

$$\textit{Wt of Salt required} = \textit{Intended solution concentration (g } l^{-1}\textit{)} \times \textit{Volume of solution} \times \frac{\textit{Formula Wt of Salt}}{\textit{Formula Wt of Metal}}$$

Table 2.9. Preparation of Hydroponic Metal Solutions

Inorganic Salt	Intended Metal Concentration mg l^{-1}	Required weight of Salt (g)	Actual Weight of Salt (g)	Actual solution Metal Concentration mg l^{-1}
Zinc Chloride	10 100 500	0.3328 3.328 16.64	0.3409 3.3605 16.6705	10.2 101 501
Cadmium Chloride	10 100 500	0.3248 3.248 16.24	0.3457 3.2555 16.2555	10.6 100.2 502
Nickel Chloride	10 100 500	0.648 6.48 32.4	0.666 6.4832 32.4456	10.3 100 501
Copper Chloride	10 100 500	0.3376 3.376 16.88	0.3537 3.4919 17.0292	10.5 103 504
Potassium Dichromate	10 100 500	0.4512 4.512 22.56	0.4701 4.526 22.6085	10.4 100.3 501
Lead Nitrate	10 100 500	0.256 2.56 12.8	0.2693 2.5930 12.8506	10.5 101 502

The actual weight of salts shown in Table 2.9 were dissolved in approximately 900 ml of deionised water and made to 1 litre. The 1 litre of prepared solution was added to 15 litres of nutrient solution to give the required solution concentration.

2.7.3 Experimental Procedure

The selected tree seedlings were arranged into their respective containers. The containers were filled with 16 litres of quarter strength nutrient solution and the seedlings were left for a two week period. The two week period was required to allow the trees to acclimatise to their new environment and to develop new roots appropriate for growth in nutrient solutions. During the acclimatising period the water levels in the pots were topped up with deionised water. The day length was maintained at 16 hours, using a mercury lamp and timer. No attempt was made to control temperature, although

automated greenhouse window openers were used as a means of controlling the temperature in the greenhouse.

Prior to the addition of the prepared heavy metal solutions to the nutrient solutions the volume of nutrient solution was allowed to fall to 15 litres. This volume was assessed using a calibrated 'dip-stick' with pre-calibrated levels for 15 and 16 litres respectively.

The prepared 1 litre metal solutions were added to the respective treatment tanks. Time zero of the experiment was taken when the metal salt was added to the nutrient solutions. The solutions were aerated for a period of time following the addition of metal salts to help mix the solutions. Throughout the experiment the day length was maintained at 16 hours. The nutrient solution volumes were monitored daily and the volumes topped up to 16 litres with deionised water.

The metal solutions for all treatments were added on the 5 June 1995, during the following period foliage samples were collected, where possible from all study trees.

2.7.4 Sampling and Analysis

Foliage samples were collected on day 1, day 4 and day 7 after the addition of metal salts. Due to the volume of biomass samples to be segregated, sorted and dried at the end of the experiment it was impossible to harvest all the vegetation on one date so the final sampling date varied for different treatments, however all plants within the same treatment were harvested at the same time.

Leaf samples were collected from all species at all metal treatments on day 1, but from only willow and poplar species on day 4. The full compliment of leaf samples were collected on day 7. It should be noted some samples were not collected due to the lack of green foliage. At the end of the experiment some 2 to 4 weeks after the addition of the metal salts whole seedlings were harvested and segregated into root, stem and foliage. Details of the final sampling periods are included in Table 2.10.

Table 2.10. Final Harvesting (Hydroponics)

Period to final Sampling	Treatment Sampled
Day 15	500 Ni
Day 16	10 Ni, 100 Ni
Day 18	10 Cu, 100 Cu, 500 Cu
Day 21	10 Cr, 100 Cr, 500 Cr
Day 30	10 Cd, 100 Cd, 500 Cd
Day 31	10 Pb, 100 Pb, 500 Pb 10 Zn, 100 Zn, 500 Zn

The samples were segregated into leaf, twig/stem and roots. The roots were rinsed in deionised water to remove surface metal contamination prior to oven drying. The twig/stem samples from poplar and willow were visually segregated into old (brown) and fresh (green) material. All samples were oven dried and prepared in accordance with procedures provided in Section 2.2.3.

Prior to the collection of biomass samples from the nutrient culture experiment the health of all tree species were recorded. The results of the visual assessment are included in Chapter 5.

The oven dry and ground biomass samples were digested in acid and analysed by atomic adsorption techniques in accordance with detailed procedures provided in Section 2.4. The metal analysis results are presented and discussed in Chapter 5.

CHAPTER 3
FIELD EXPERIMENT

3.1. INTRODUCTION

From the literature review various fields of research were covered relating to the interaction of heavy metals with vegetation. One of the most studied soil contamination issues was heavy metal contamination of soil arising from metal smelting operations (Hogan & Wotton, 1984, Hazlett et al., 1983 and Tyler 1984) and its effect on tree survival and metal uptake and the vegetation colonisation of metalliferous mine spoil wastes (Smith & Bradshaw, 1979 and Eltrop et al., 1991). These studies reported that vegetation including trees were tolerant to heavy metal concentrations in soil to maximum levels of 29,000 mg kg^{-1} lead, 20,000 mg kg^{-1} copper, 12,000 mg kg^{-1} nickel and 10,000 mg kg^{-1} zinc. In addition the evolution of tolerance in vegetation to heavy metal contamination was discussed by MacNair (1987), Baker (1987), Dickinson et al. (1991) and Wilkins (1991) and was reported as a factor of genetically determined natural selection.

The ability of trees to take-up and hold nutrients and metals within biomass has also been recognised in various studies. Tree biomass has been widely studied as a means of environmental dating by Stewart (1966), Stewart et al. (1991), Rolfe (1974), Barnes et al. (1976) and Cutter & Guyette (1993). The growth rings of trees were studied to determine chronological changes and fluctuations of metals in the environment. These studies also observed that metal concentrations were higher in outer growth rings of trees when compared with concentrations in heartwood (Okada et al., 1993a and 1993b, Queirolo, 1990). Young (1971) reported the concentration ratio of metals in bark to stem was in the order of 4.6:1.

The seasonal variation of nutrients and trace elements with forests have been studied and reported by Fromm et al. (1987) and Wyttenbach & Tobler (1988). These studies have shown that nutrients and trace metal concentrations can vary significantly through the growing season, some metals increase in concentration while others fall.

It is also known that soil contamination can be an economic burden to redevelopment and that phytoremediation offers potential cost benefits, provided suitable plant

species can be identified to carry out remediation works within a feasible time scale (Department of the Environment, 1991, Cunningham & Berti, 1993 and Stomp et al., 1993 and 1994). Guidance is also available for interpreting at what concentrations metals in the soil are likely to be a risk to development (ICRCL 89/83 and Dutch Guidelines, 1994).

There are many experimental procedures which could be devised to study heavy metal uptake by vegetation. This Chapter presents the findings of a study titled 'Field' experiment and will discuss the findings of investigations undertaken to determine the uptake of heavy metals from soil by woodland species growing on derelict and contaminated soil.

The results are the accumulation of a two year study of selected trees which were found to be growing on derelict land. Site investigations and contamination testing confirmed that the soils at the site contained elevated concentrations of heavy metals, including copper, chromium, lead and zinc.

3.2 SELECTION OF SITES

Glasgow University department of Agricultural Food and Environmental Chemistry were previously commissioned by Central Region Council to undertake intrusive site investigations at two sites in Central Scotland near Falkirk. The findings of the investigation and the chemical test data confirmed both sites were contaminated with elevated concentrations of heavy metals. Based on the test results and a general knowledge of the site , selected areas within both sites were preliminary identified as potential study areas. A site visit and site walkover were arranged to inspect the sites and identify suitable tree species growing within the selected areas.

The two sites were named Redding and Summerford. Redding was located approximately 3 km to the south east of Falkirk Town Centre and Summerford was located approximately 2 km to the west of the Town Centre.

The Redding site was historically used for explosive manufacturing and was operated by the Nobel Explosive Company until its closure. The Summerford site was formerly occupied by a chromium works owned by Falkirk Chemical Company. Both sites were derelict and undeveloped and were supporting a number of tree species including birch (*Betula pendula*), willow (*Salix caprea L.*) and sycamore (*Acer pseudoplatanus*). The location of the sites are shown on the site location plan (Figure 3.1).

The sites contained elevated soil metal concentrations as defined with reference to guidance levels documented in the Interdepartmental Committee for the Redevelopment of Contaminated Land (ICRCL Guidance Note 89/83). That is to say some of the metal concentrations in the soils collected from both site were in excess of threshold values for playing fields and open space.

The field study experiment comprised the sampling and analysis of biomass samples collected from selected trees growing at both sites. The biomass samples were collected at monthly intervals over a period of two years (March 1994 to March 1996).

DRAWING TITLE	DRAWING No.	SCALE
SITE LOCATION PLAN	**FIGURE 3.1**	**1:50000**

3.2.1 Redding (Former Nobel Explosive Factory)

3.2.1.1 Location

The site is located approximately 3 km to the south east of Falkirk Town Centre. The grid reference to the centre of the site is NS 914 779 and the site area extends to approximately 21 hectares. The northern boundary of the site adjoins the south bank of the Union Canal which runs east to west. The site boundary to the south adjoins the village of Redding Muir. A disused tip and Polmont Young Offenders Institution is located to the east beyond the site boundary. The western boundary adjoins open fields which are in agricultural use.

3.2.1.2 Site Description

The site is irregular in shape and is heavily wooded with a mix of deciduous and coniferous tree species. The site topography slopes down to the north, towards the canal. The site is intersected with a number of meandering tracks and derelict two storey brick buildings are sparsely dotted over the site, some of which are enclosed within earth bunds. There is also a number of concrete and open drainage channels which flow to the canal.

An existing industrial estate occupies the northern extent of the previous explosive works site adjacent to the canal.

3.2.1.3. Historical Development

The history of the site has been compiled with reference to historical ordnance survey sheets, published information and other anecdotal information made available during the study. The following information relates the history of explosive manufacture at the site.

In early 1873 the British Dynamite Company formed and a factory was built at Ardeer on the Ayrshire coast, subsequently in 1877 Nobel Explosive Company acquired the factories at Westquarter and Redding Moor for the manufacture of detonators and mercury fulminate. The development of the site and the processes undertaken were dictated by development of the detonator.

The first detonator was a glass tube containing a small charge of gun powder which, was imbedded in a charge of nitroglycerin. Further developments produced a detonator which comprised fulminate of mercury contained in a copper cap. As demand for detonators increased so did the size of the factory. In 1877 the factory occupied 4.5 acres and employed six staff; in 1909 the factory occupied 15 acres and employed 350 staff; in 1914 the factory occupied 19 acres and employed 450 and by 1918 it covered 45 acres with 800 staff. This expansion was attributed to increased detonator demand due to the First World War. In the period 1936 to 1938 most of the plant was dismantled and transferred to Ardeer, although the mercury fulminate plant was retained at Redding. The Second World War (1939 -1945) brought new demands, the production of fulminate of mercury and sodium azide was resumed at Redding. At its peak the operations employed 1700 staff working three shifts.

After the war the factory was involved with research and development of plastic covered conducting wire while maintaining the production of high quality specialised electric detonators for export.

The first detonators were charged with fulminate of mercury mixed with potassium chlorate in a copper tube. Later tetryl (2:4:6 trinitrophenyl methyl nitramine) which was used to fill the lower section of a copper tube along with fulminate of mercury.

Further detonator developments included:

- aluminium tubes replaced copper tubes
- in 1920 lead azide replaced mercury fulminate as the initiating charge
- in 1923 lead azide was replaced by a mixture of lead azide and styphanate (known as lead trinitroresorcinol)

- between 1920-1936 lead mononitroresorcinol replaced copper acetylide as the main ingredient of electric fuse heads

A summary of the chemical compounds used and manufactured at the site are given below:

Fulminate of mercury was prepared by dissolving mercury in strong nitric acid and precipitating in alcohol. The precipitate was filtered and washed until free of acid. The mercury was probably supplied as the liquid metal and was unlikely to have been extracted at site.

Mercuric fulminate

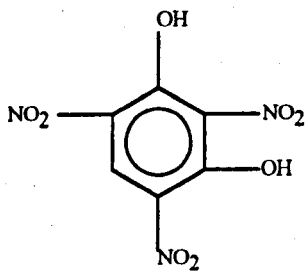
$$Hg(C{=}N{-}O)_2$$

Lead Azide

$$Pb(N_3)_2$$

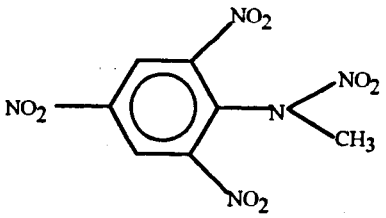
Lead azide was manufactured by reacting lead acetate with sodium azide.

Styphanate



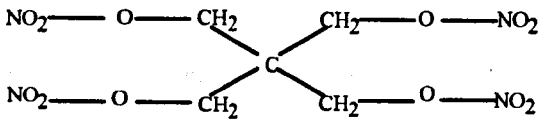
Lead styphanate was made by reacting magnesium styphanate with lead acetate.

Tetryl (2:4:6 trinitrophenyl methyl nitramine)



Tetryl was prepared by the nitration of dimethyl aniline in concentrated sulphuric acid.

PETN (pentaerythritol tetranitrate)



3.2.1.4. Site History (Ordnance Survey)

The historical development of the site was also determined by examining past editions of Ordnance Survey (OS) sheets relevant to the site held by Falkirk Library. Summary details of the plans inspected are given below and extracts of the sheets are included (Figure 3.2 to 3.6).

Plan	Date	Scale
Stirlingshire XXX.8	1860	1:2500
Stirlingshire XXX.8	1897	1:2500
Stirlingshire XXX.8	1918	1:2500
NS 9077/ NS 9177	1964	1:2500
NS 97 NW	1969	1:10560

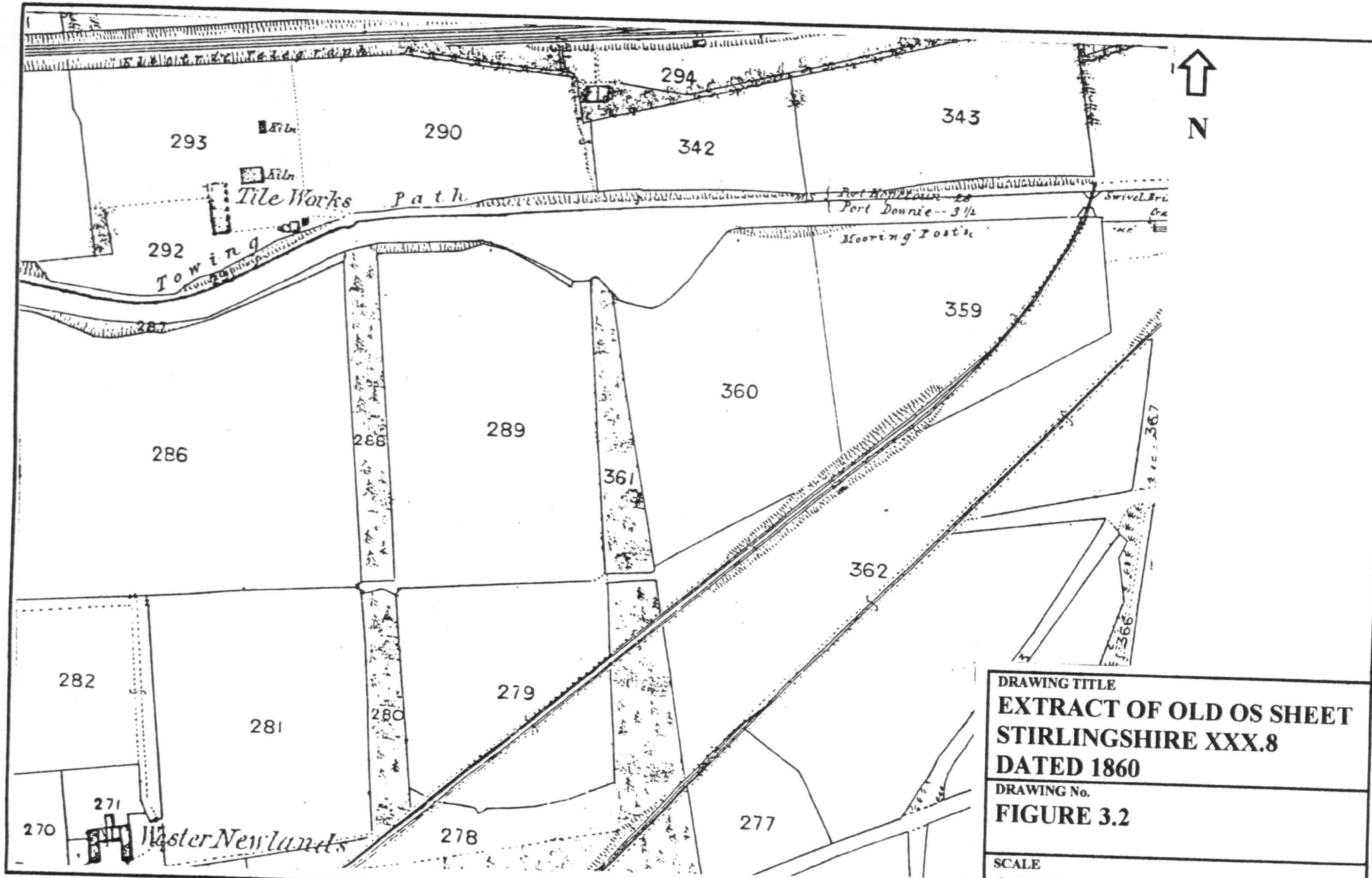
The first edition indicates that the Union Canal existed in its present location prior to 1860. The site in undeveloped and consists of agricultural fields. A tile works is located to the north west of the site on the north bank of the canal and two railway

lines are evident to the south west of the site. Wester Newlands farm is located to the south east of the site.

The plan dated 1897 only covers the northern extent of the site but there would appear to be no development within the site. Redding Colliery is established to the north east of the site beyond the canal along with a number of rail lines. A small boat house has been constructed on the canal basin.

The plan dated 1917 indicates that no development has occurred to the south of the canal within the site, however, the 'Explosive' works are extensive to the north of the canal. No other significant changes are evident.

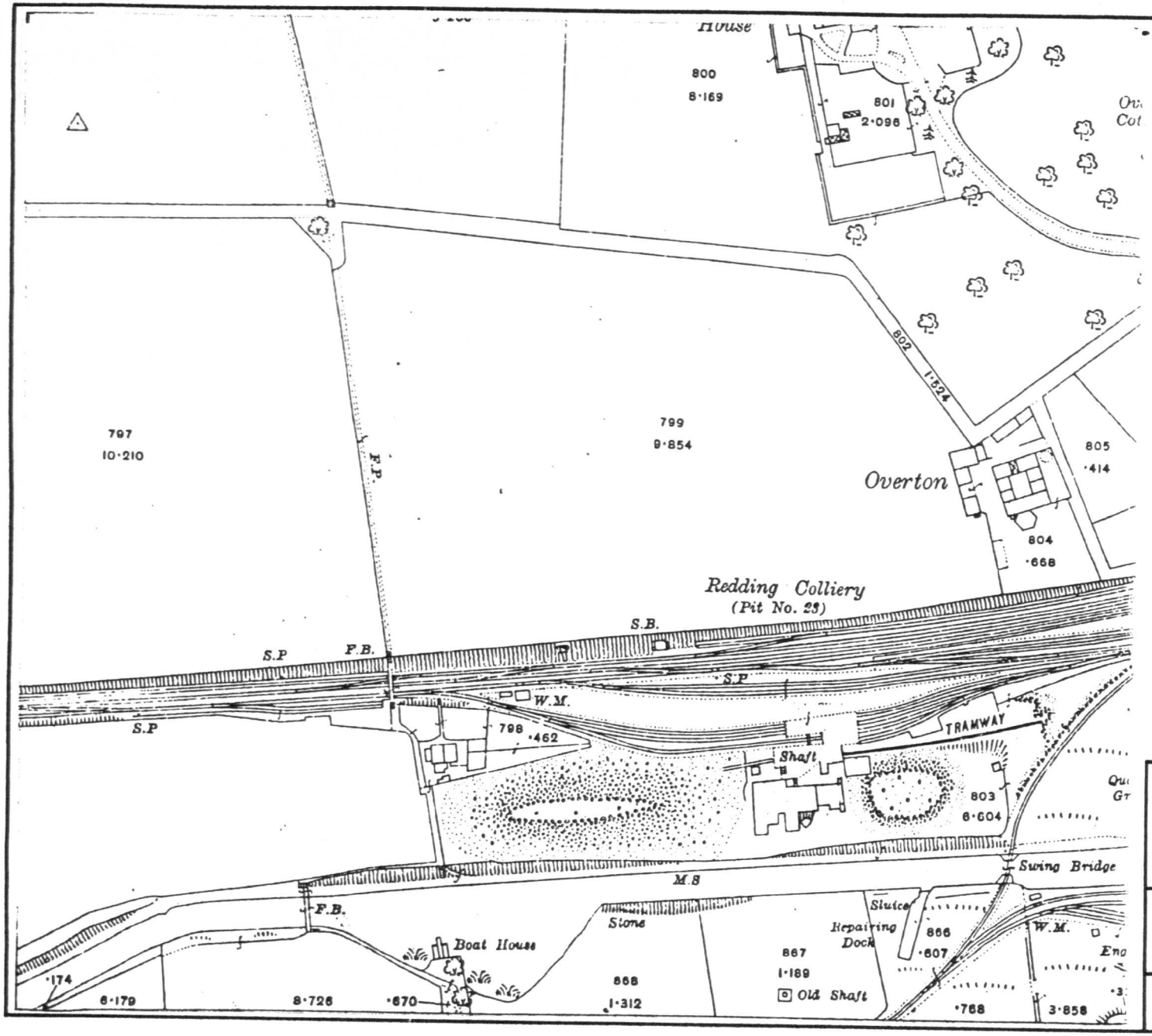
The plans dated 1964 and 1969 indicate that the explosive works have extended to the south and occupy a larger area to the south of the canal than to the north. The factory building are isolated within bunded areas and are linked by a network of connecting paths. The Redding Colliery no longer exists and a 'tip' is evident to the east of the site with the Young Offenders Institution beyond.



DRAWING TITLE
EXTRACT OF OLD OS SHEET STIRLINGSHIRE XXX.8 DATED 1860

DRAWING No.
FIGURE 3.2

SCALE
1:2500



DRAWING TITLE

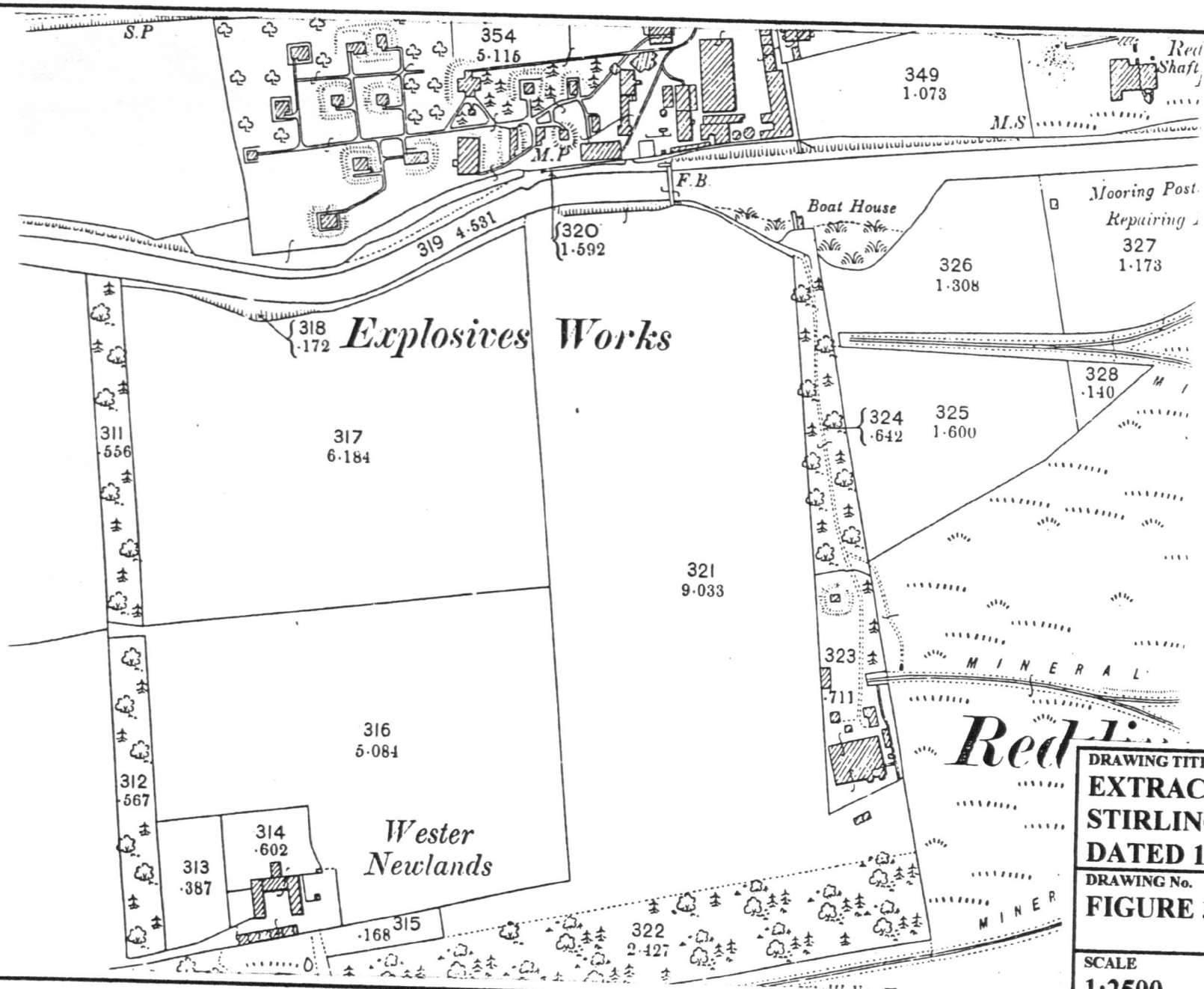
EXTRACT OF OLD OS SHEET STIRLINGSHIRE XXX.8 DATED 1893

DRAWING No.

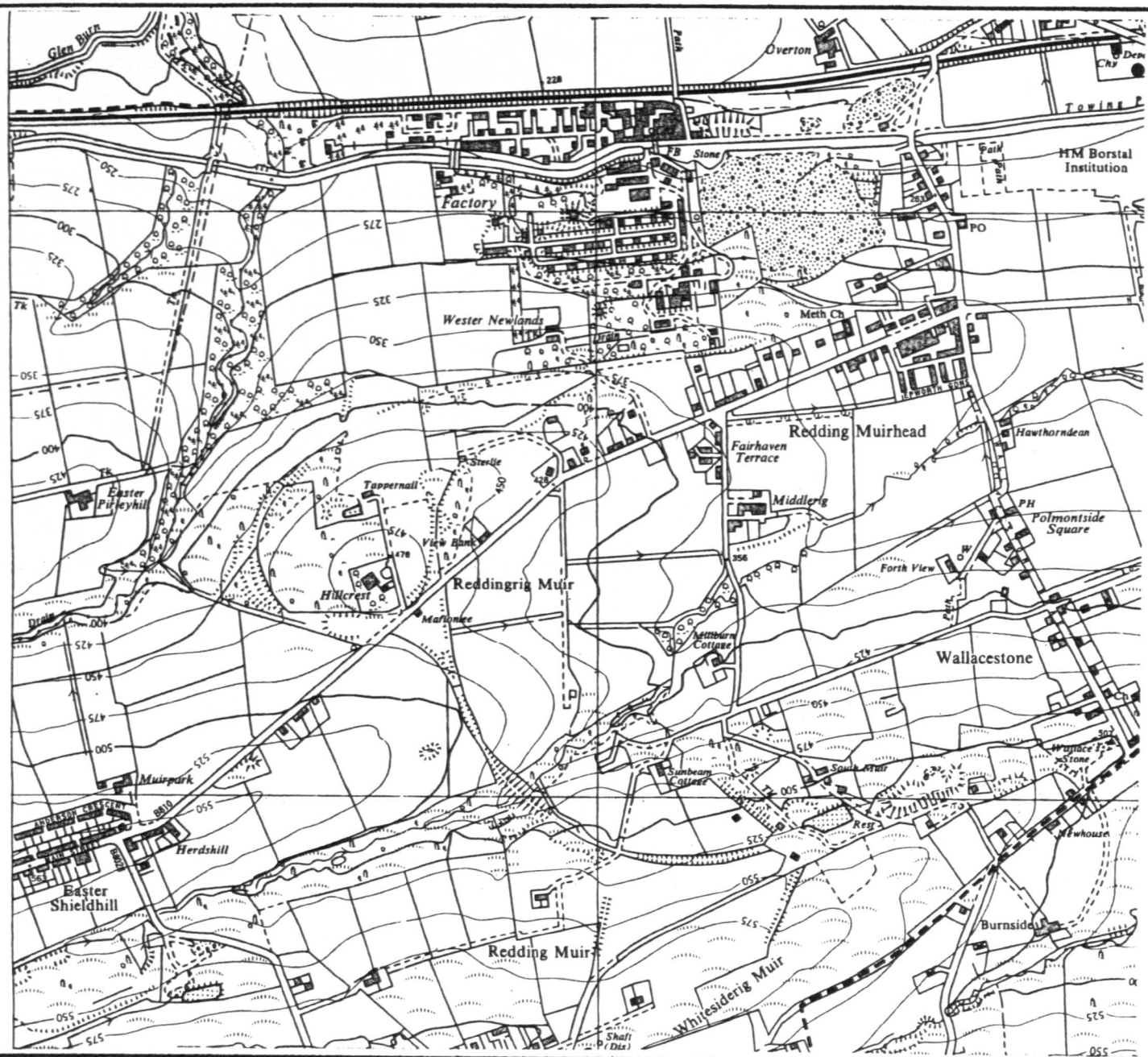
FIGURE 3.3

SCALE

1:2500



DRAWING TITLE	EXTRACT OF OLD OS SHEET STIRLINGSHIRE XXX.8 DATED 1917
DRAWING No.	FIGURE 3.4
SCALE	1:2500



N

DRAWING TITLE

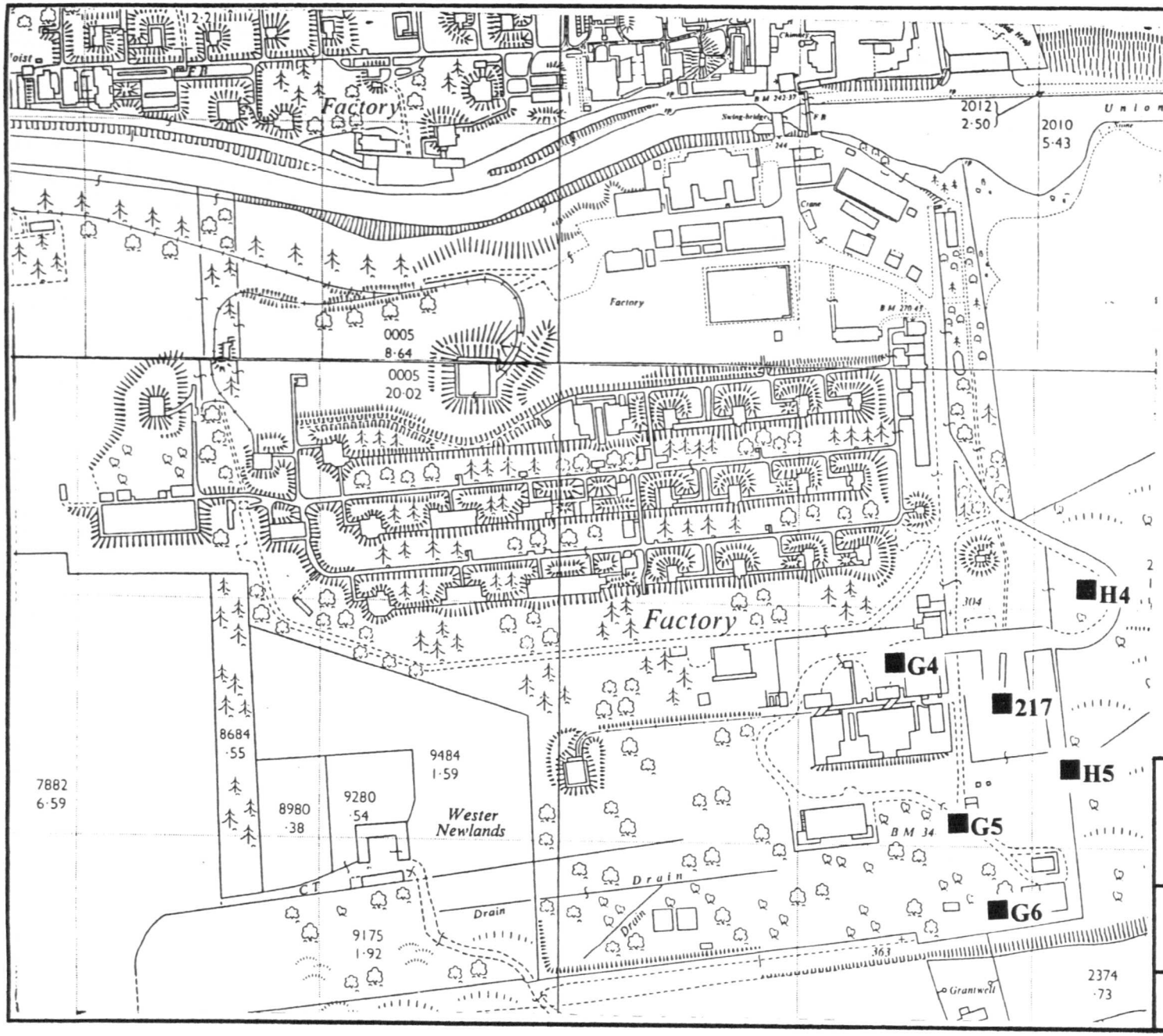
EXTRACT OS SHEET
NS 97 NW
DATED 1969

DRAWING No.

FIGURE 3.5

SCALE

1:10560



DRAWING TITLE

EXTRACT OS SHEET NS 9077/NS 9177 DATED 1964

DRAWING No.

FIGURE 3.6

SCALE

1:2500

3.2.1.5. Contamination

Given the former operations undertaken at the site and the nature of the chemicals used and produced the potential for heavy metal contamination was considered to be high.

The intrusive investigation and contamination assessment undertaken by The University of Glasgow concluded that areas of the site were contaminated with elevated concentrations of copper, nickel, zinc, mercury and lead. The levels recorded were found to be in excess of ICRCL threshold values. A selection of the chemical test results obtained by the University of Glasgow are included in Table 3.1 and the locations from which the samples were collected are shown on Figure 3.6 The maximum concentrations of copper, nickel, zinc, mercury and lead exceeded the ICRCL threshold values by 111500%, 311%, 1500%, 9850% and 985% respectively.

Table 3.1. Results of Previous Site Investigation, Redding

SITE	Depth cm	% Stones	pH	LOI	Cu ($mgkg^{-1}$)	Ni ($mgkg^{-1}$)	Zn ($mgkg^{-1}$)	As ($mgkg^{-1}$)	Cd ($mgkg^{-1}$)	Cr ($mgkg^{-1}$)	Hg ($mgkg^{-1}$)	Pb ($mgkg^{-1}$)
G4A	0-30	32	8.4	8.9	628	195	238	2.0	0.3	56	235	438
G4B	75-125	3	7.4	6.0	81	31	49	0.8	<0.2	47	30	20
G5A	0-40	30	7.5	18.8	1240	218	233	1.5	<0.2	64	1030	125
G6A	0-15	32	6.0	20.6	442	85	104	2.9	0.3	59	1970	291
G6B	15-80	5	6.4	8.4	24	22	32	1.5	<0.2	41	360	48
H4A	0-10	0	6.7	64.1	145000	20	4500	0.8	1.7	14	65	19700
H4B	10-40	24	8.4	20.0	19800	81	1550	0.7	0.5	61	6	973
H4C	40-50	5	7.4	6.5	109	48	100	0.8	<0.2	51	15	44
H5A	0-10	16	6.8	24.7	33	35	55	1.1	<0.2	34	4	33
H5B	10-110	28	6.9	33.3	44	34	30	1.5	<0.2	36	<1	25

3.2.2 Summerford (Former Chromium Works)

3.2.2.1. Location

The site is located approximately 2 km to the west of Falkirk Town Centre and extends to an area of approximately 11.5 hectares. The site is bounded to the south by the main Edinburgh to Glasgow railway and to the north by an unsurfaced track (Summerford Road) with parkland and multi-storey residential buildings beyond. The site is located within a residential area. A new housing development has been established on the north west corner of the site and a nursing home (Summerford House) is located in the north-east corner of the site.

The location of the site is shown on the site location plan (Figure 3.1).

3.2.2.2. Site Description

The site is triangular in shape and supports a cover of rough grassland and trees. The site topography slopes down from the railway line in the south towards Summerford Road. The site is enclosed by a wire fence and signs identify the site as 'Toxic-Keep Out'. It is understood the slope is comprised of deposited waste from the chromium works.

3.2.2.3. Historical Development

The historical development of the site has been assessed with reference to historical Ordnance Survey (OS) sheets and other historical information provided during the course of this project. Summary details of the plans inspected are given below and extracts of the sheets are included (Figure 3.7 to 3.13).

Sheet	Date	Scale
Stirlingshire XXX.2/XXX.3	1860	1:2500
Stirlingshire XXX.2/XXX.3	1897	1:2500
Stirling XXX.6	1917	1:2500
Stirling XXX.6	1947	1:2500
NS 8679 and NS 8779	1961	1:2500
NS 8679 (Superplan)	1995	1:2500

A factory identified as 'Chemical Works' is evident on the first edition OS sheet and is located in the west of the site. The railway line is located to the south of the site and a canal link is formed between the Union Canal and the Forth and Clyde Canal to the west of the site. Summerford House and Glenfuir House are situated to the north with the Union and Camelon Foundry beyond.

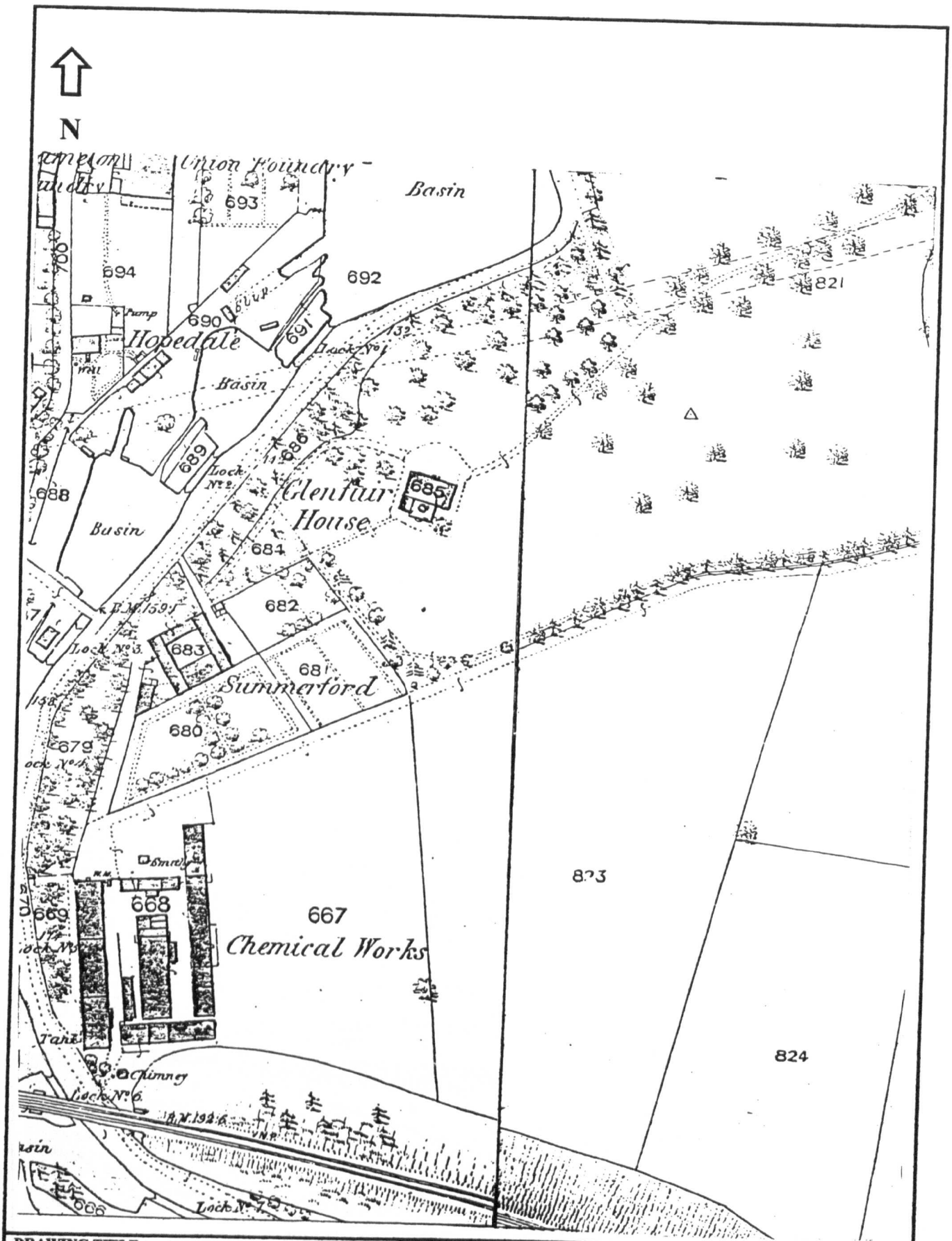
The OS sheet dated 1897 identifies the previous building within the site as 'Falkirk Chemical Works' These works and Camelon Chemical Works located to the north are serviced by a rail network. The beginnings of a spoil bing is evident, located in the south of the site.

A second works building has been established in the north east corner of the site on the OS sheet dated 1917 and the spoil mound has increased in size. This new building is identified as Summerford Iron Works on the OS sheet dated 1947. Falkirk chemical works appear to have closed and the spoil bing has increased in size.

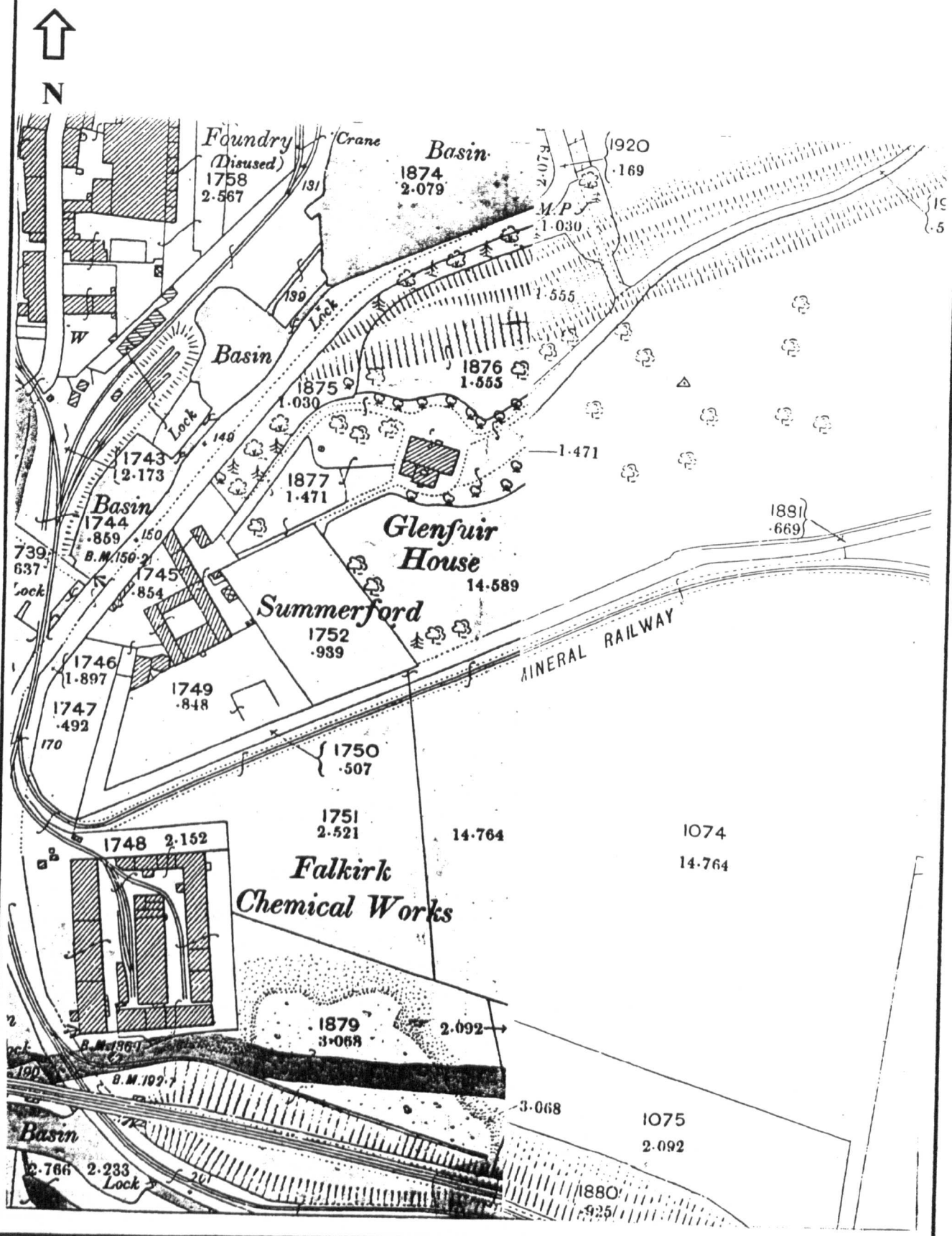
The OS sheet dated 1961 show no significant change in land use. The canal link and basins have been infilled and the buildings in the west of the site are identified as a ruin. The OS sheet dated 1995 shows the present site layout. The residential development 'Summerford Gardens' is present to the west and Summerford House occupies the north east corner.

A general summary of the historical use of the site was provided by Central Region and is summarised as follows. Falkirk Chemical Company established a chrome works at the site in the period between 1850 and 1870. The works were primarily involved in supplying chromium to the pigment and tannery industries which included the ICI dye works at Grangemouth. The chrome works closed in 1932.

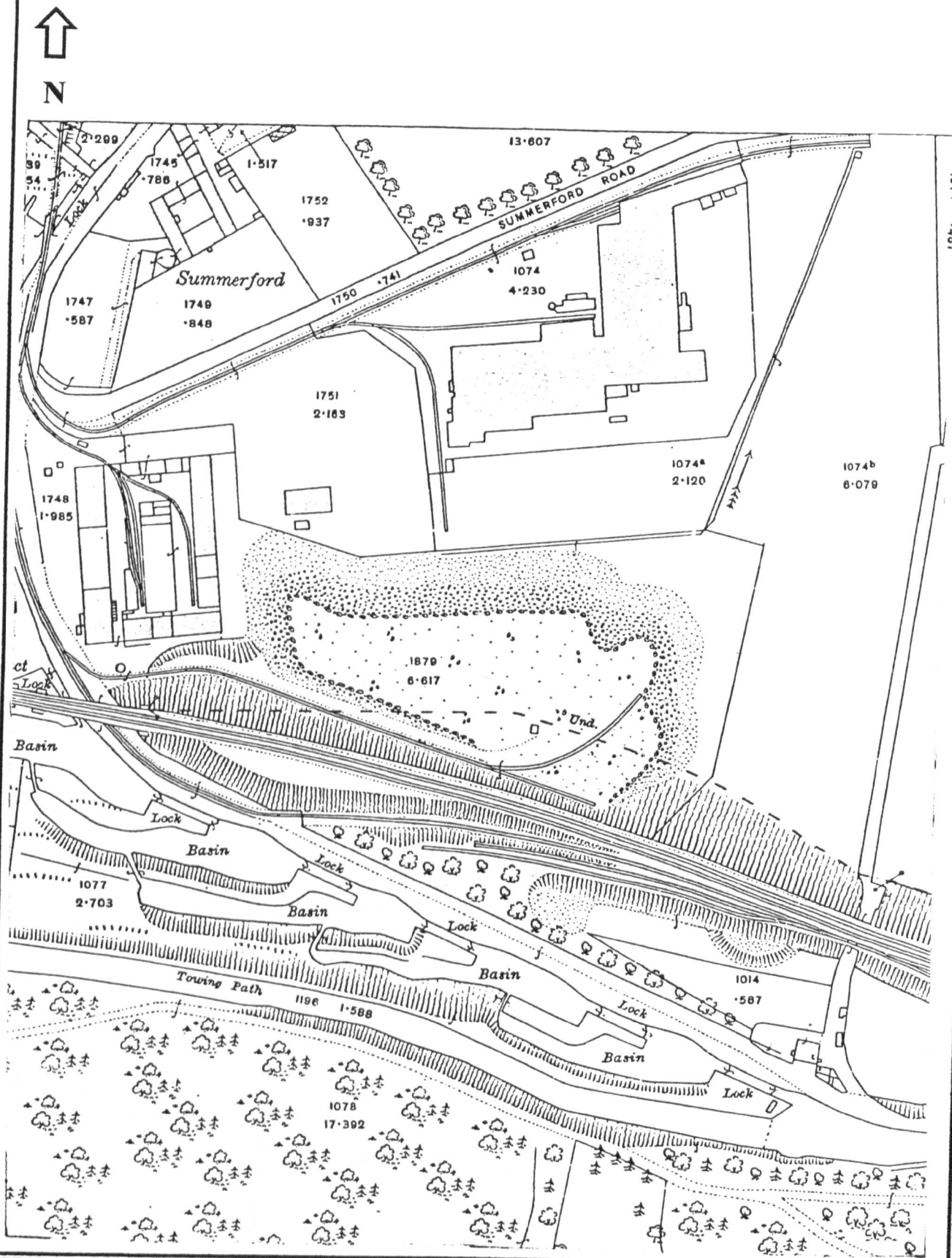
Miller's Pre-cast Concrete was established in the east corner of the site, however, the company went into liquidation in the late 1970's. In an attempt to 'tidy' the site Central Region demolished the buildings and the bing material was spread over the demolition waste and landscaped. Unknown at the time, the bing material comprised waste from the chromium works. Central Region realising the potential risk from this contamination, established vegetation on the site to reduce the potential for dust generation. The site was fenced and warning signs were erected to warn the public of the contamination.



DRAWING TITLE	DRAWING No.	SCALE
EXTRACT OF OLD OS SHEET STIRLINGSHIRE XXX.2/3 DATED 1860	**FIGURE 3.7**	**1:2500**

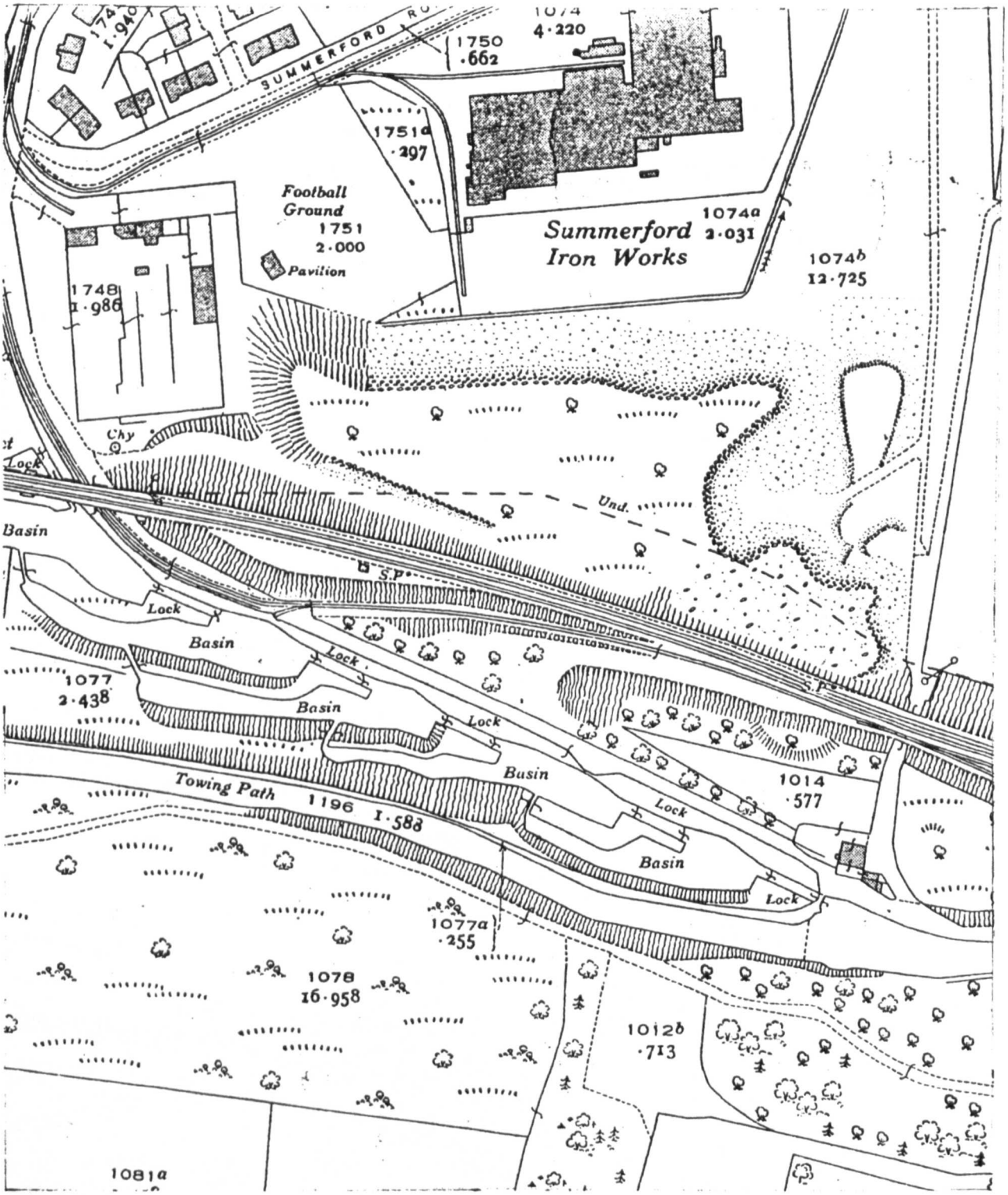


DRAWING TITLE	DRAWING No.	SCALE
EXTRACT OF OLD OS SHEET STIRLINGSHIRE XXX.2/3 DATED 1897/1898	**FIGURE 3.8**	**1:2500**

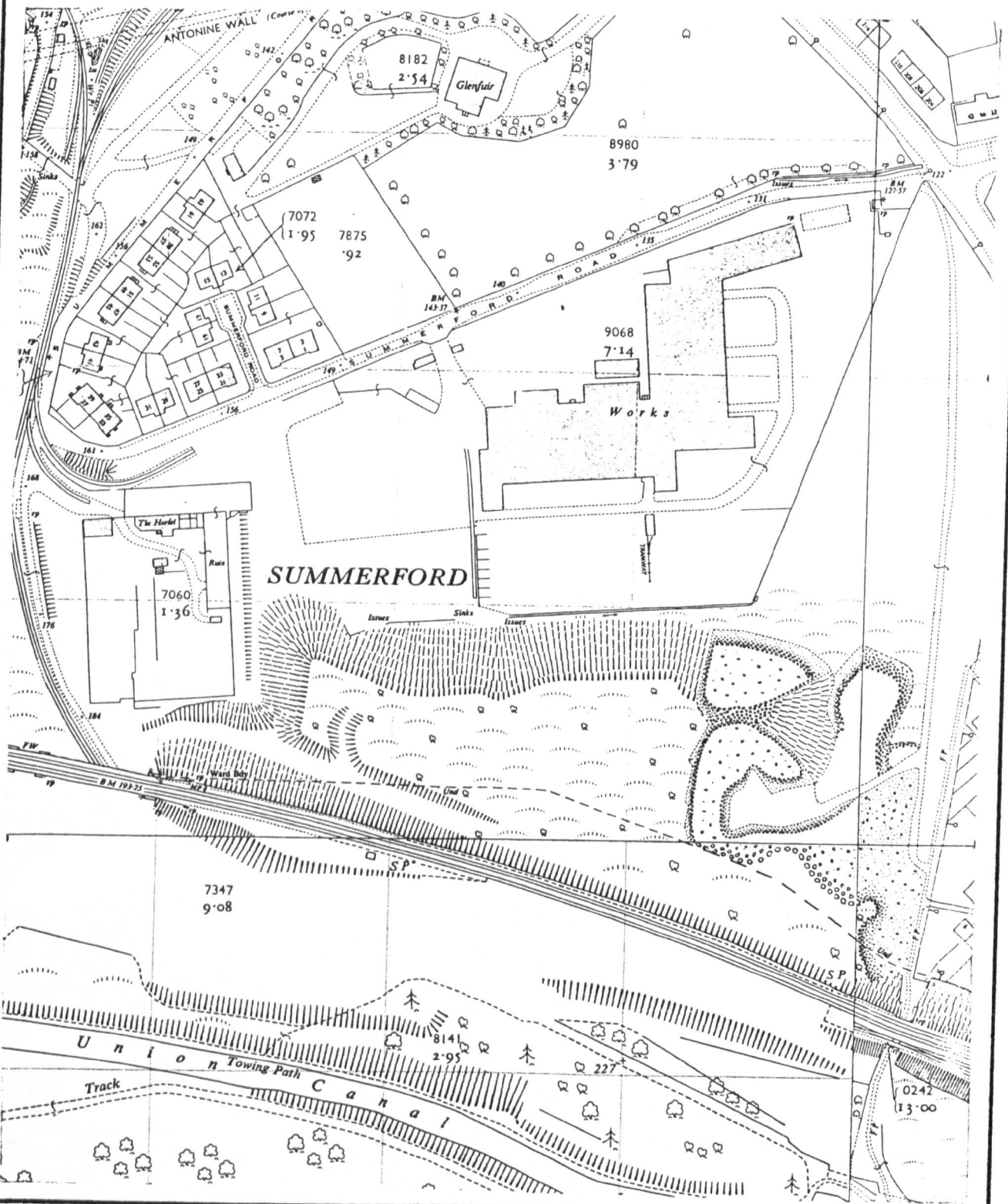


DRAWING TITLE	DRAWING No.	SCALE
EXTRACT OF OLD OS SHEET STIRLINGSHIRE XXX.6 DATED 1917	**FIGURE 3.9**	**1:2500**

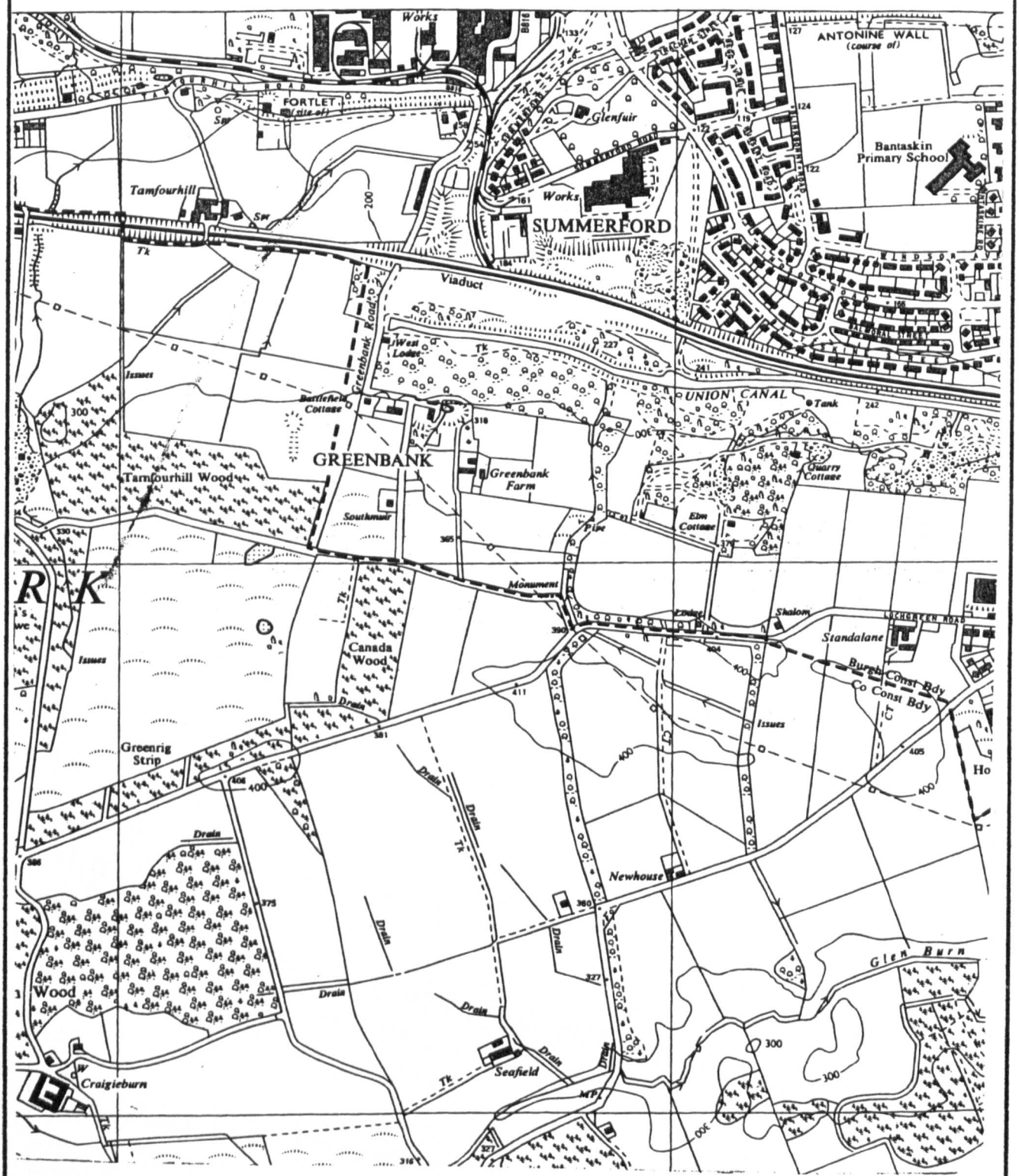
N



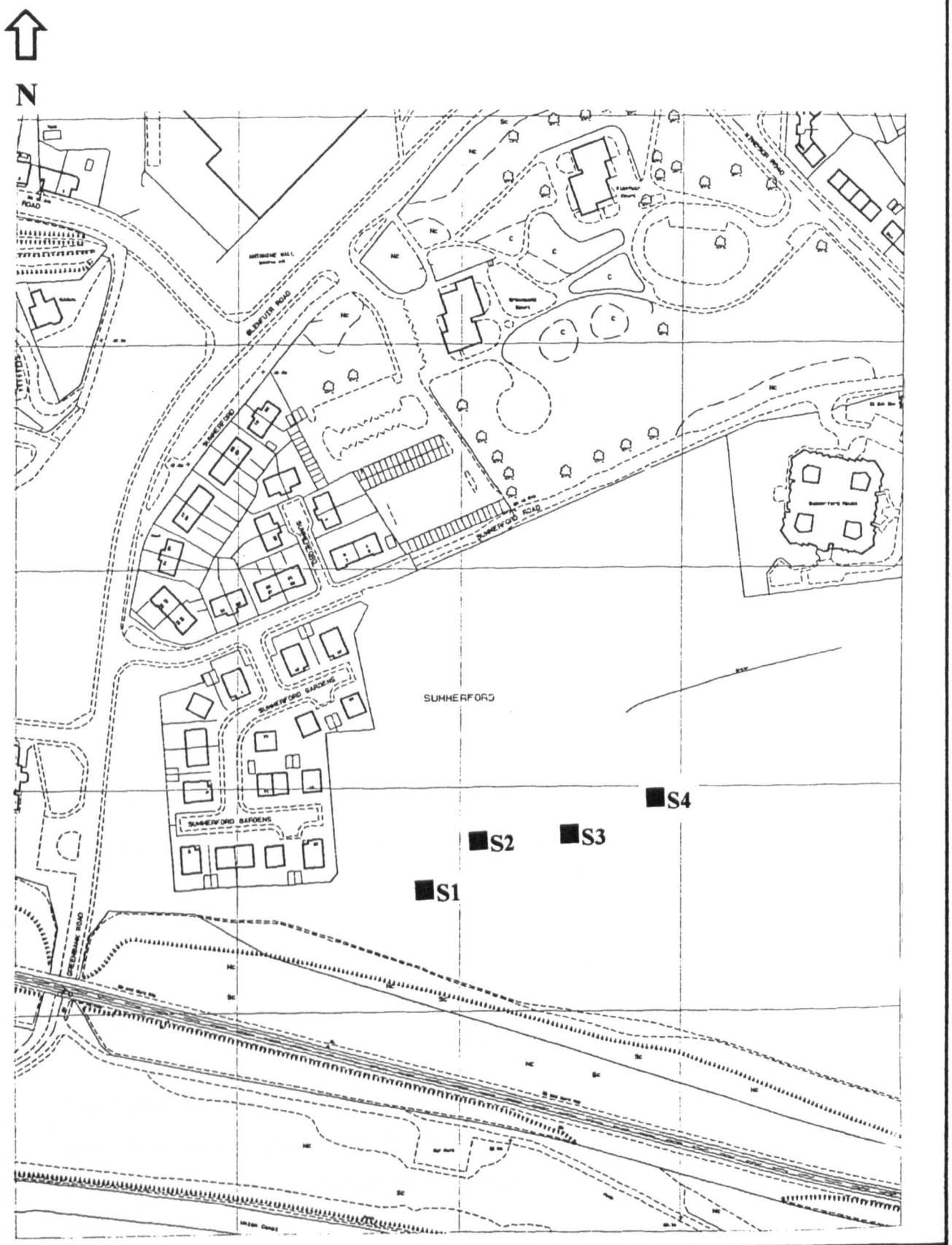
DRAWING TITLE	DRAWING No.	SCALE
EXTRACT OF OLD OS SHEET STIRLINGSHIRE XXX.6 DATED 1947	**FIGURE 3.10**	**1:2500**



DRAWING TITLE	DRAWING No.	SCALE
ORDNANCE SURVEY SHEET NS 8679/NS 8779 DATED 1961	**FIGURE 3.11**	**1:2500**



DRAWING TITLE	DRAWING No.	SCALE
ORDNANCE SURVEY SHEET NS 87 NE DATED 1967	**FIGURE 3.12**	**1:10560**



DRAWING TITLE	DRAWING No.	SCALE
SUPERPLAN NS 8679 DATED 1995	**FIGURE 3.13**	**1:2500**

3.2.2.4. General Processes

The main waste products arising from chromium works are potassium chromate and potassium dichromate (Liptrot, 1978). The main ore of chromium is chromite ($FeCr_2O_4$). Finely ground chromite was mixed with sodium carbonate and roasted in an oxidising atmosphere. The sodium chromate was soluble in hot water leaving insoluble ferric oxide. The sodium chromate is converted to sodium dichromate by acidifying the solution with sulphuric acid solution and sodium dichromate is reduced by carbon. The chemical reactions can be summarised as follows:

- $FeCr_2O_2 + 8Na_2CO_3 + 7O_2 \rightarrow 8Na_2CrO_4 + 2Fe_2O_3 + 8CO_2$
- $2Na_2CrO_4 + H_2SO_4 \rightarrow Na_2Cr_2O_7 + Na_2SO_4 + H_2O$
- $Na_2Cr_2O_7 + 2C \rightarrow Cr_2O_3 + Na_2CO_3 + CO$
- $Cr_2O_3 + 2Al \rightarrow Al_2O_3 + 2Cr$

Ferric oxide is the waste material from the chromium works and contains in the order of 1-3% chromium which was historically dumped in spoil mounds.

The potassium dichromate produced can be used in a number of industrial processes including the preparation of chrome alum. Chrome alum is used as a mordant in dyeing and for tanning leather.

3.2.2.5. Contamination

The Summerford site had not been subject to an extensive site investigation, however a number of soil samples previously collected from the site for undergraduate projects contained elevated concentrations of chromium. These results were not available for inclusion.

3.3 PRELIMINARY SITE INVESTIGATIONS

3.3.1 Introduction

Summerford and Redding were considered to be appropriate study sites for the purpose of the experiment. The sites were located within close proximity to residential areas and there when no plans for redevelopment at the time of this study. The sites were also supporting an established tree population. The sites were 'typical' of derelict sites where redevelopment would have been undertaken had it not been for the levels of contamination and the prohibitive costs of dealing with the contamination.

In January 1994 a site visit and site walkover inspection of the Summerford and Redding was made to:

- select potential study areas within each site
- select potential study trees within study areas.

At the time of the site walkover a number of representative shallow soil samples were collected from each site.

3.3.2 Procedure

The soil samples were collected from shallow hand excavated trial pits in accordance with the procedures documented in Chapter 2, Section 2.1.2.

A total of four soil samples were collected from the Summerford Site. The hand excavated pits were located on the west side of the spoil mound within a stand of birch trees. The indicative location of the collected samples are shown on Figure 3.13.

Seven soil samples were collected from shallow hand excavated trial pits at Redding, comprising three soil samples from area H4, two soil samples from area 217 and a further two samples were collected from area G5. The indicative location of the collected samples are shown on Figure 3.6.

The collected soil samples were prepared in accordance with the procedures documented in Chapter 2, Section 2.1.4 and were analysed for residual metal in accordance with the procedures documented in Section 2.3.9.

3.3.3 Results

The laboratory test results obtained from the preliminary site investigation are tabulated in Table 3.2. The theoretical limit of quantification and more importantly variation of the analytical results are discussed in Chapter 2, Sections 2.5.4 and 2.5.5 respectively. The reproducibility of the metal analysis studies is ± 7, 3, 5 and 10 mg kg^{-1} for zinc, copper, lead and nickel.

Table 3.2. Results of Preliminary Site investigation

Sample	Metal concentrations in soil (mg kg^{-1})				
	Chromium	Lead	Zinc	Copper	Nickel
Summerford 1	1840	326	66	109	99
Summerford 2	1570	418	81	123	100
Summerford 3	1700	295	70	86	91
Summerford 4	2060	358	75	99	109
Redding H4-1	27	2798	73	1812	121
Redding H4-2	24	1660	76	337	206
Redding H4-3	15	5640	827	7310	3
Redding 217-1	18	2420	70	1080	135
Redding 217-2	22	640	144	783	160
Redding G5-1	20	830	55	127	53
Redding G5-2	22	2120	77	3230	207

Note: All values are expressed as mg kg^{-1} of air dry soil

3.3.4 Discussion

- **Interpretation Of Analytical Data**

The residual metal content of soil is a measurement frequently used to identify whether a soil is contaminated or not. However, the concentration of a metal in a soil can vary widely, even in natural soils making it difficult to determine at what concentration a metal becomes a hazard and therefore whether or not a soil is defined as contaminated.

In an attempt to assess soil metal concentrations obtained from laboratory analysis, published guidance levels were consulted wherever applicable. The preliminary soil metal concentrations have been assessed with reference to the following guidelines.

- Interdepartmental Committee on the Redevelopment of Contaminated Land (ICRCL) Guidance Note 59/83.

The ICRCL guidelines are advisory notes issued by the Department of The Environment which provide information on contamination levels in soil where a health hazard or environmental risk may exist. The guidelines are not statutory, although they are often consulted when contaminated land is about to be redeveloped.

The ICRCL guidelines provide different acceptable levels for redevelopment to domestic gardens and allotments; parks, playing fields and open space; and for buildings and hardstanding. The guidelines give trigger threshold concentrations and for some chemical parameters, action threshold levels. These levels vary depending on the perceived risk of each contaminant to the proposed end-use.

When the concentration of a chemical determinand is below a trigger threshold level the soil may be regarded as uncontaminated with respect to that determinand and no remediation work is required. A value above a trigger threshold indicates a potential risk. A determinand which is present at concentrations in excess of action threshold

levels indicates the soil is contaminated and the proposed landuse should be abandoned until further remedial action is taken or a change of landuse is proposed.

Reference is often made to the 'Dutch Guidelines' for chemical determinands not covered by the ICRCL Guidelines:

- The Dutch 'Intervention and Target Values - Soil Quality Standards' (Ministry of Housing, Spatial Planning and Environment, The Hague 1994).

The Dutch guidelines provide advisory levels of chemical determinands in soil similar to the ICRCL guidelines, however, the guidelines are used to classify materials on the basis of Target and Intervention values. Target values represent soil quality levels indicative of maximum concentrations in uncontaminated soil and are bench mark concentrations to be reached during decontamination works. Soil concentrations which exceed intervention values constitute serious contamination and require remedial action.

The adopted metal concentrations taken from the above guidelines and used in the assessment of the soil samples analysed are reproduced In Table 3.3.

Table 3.3. Adopted Soil Assessment Criteria

Contaminant	**ICRCL Threshold Value**	**'Dutch' Target Value**
Arsenic	40	29
Cadmium	15	0.8
Chromium (total)	1000	100
Chromium (hexavalent)	25	
Lead	2000	85
Mercury	20	0.3
Copper	130	36
Nickel	70	35
Zinc	300	140

Note: All values are expressed as mg kg^{-1} of soil

- **Previous Soil Contamination - Redding/Summerford**

The total metal concentrations at Redding provided in the 'Contamination Assessment Report' prepared by the University of Glasgow, refer Table 3.1, were determined by a nitric/perchloric acid digest of the collected soils. It should be noted this digestion procedure is similar to a nitric/hydrochloric digestion, however the procedure is often regarded more aggressive and can explode if not controlled. In this regard the nitric/perchloric procedure was not adopted for the determination of residual metal concentrations during the course of this research.

The ten soil samples were collected from areas G4, G5, G6, H4 and H5 and the approximate locations of the trial pits excavated by the University of Glasgow are detailed on Figure 3.6.

Based on the guidelines consulted a number of the soils collected from Redding contained elevated concentrations of copper, nickel, zinc, mercury and lead.

Copper was recorded to be elevated in five of the soils at concentration between 442 and 145000 mg kg^{-1}, with the highest concentration in the sample from area H4. Nickel was elevated in four samples at concentrations ranging between 81 and 218 mg kg^{-1}. Mercury was elevated in six of the samples analysed at concentrations between 30 and 1970 mg kg^{-1}, the highest concentration was in sample G6. Two samples contained elevated zinc to a maximum concentration of 4500 mg kg^{-1} in sample H4, lead was also elevated in the same sample at a concentration of 19700 mg kg^{-1}.

The maximum concentrations of copper, nickel, zinc, mercury and lead were found to exceed ICRCL trigger threshold levels by 111500%, 310%, 1500%, 9850% and 985% respectively. From this laboratory data, areas G4, G5, G6 and H4 would be considered contaminated and some form of ground remediation would be required before the site could be redeveloped.

No documented or reported soil contamination data was available for the Summerford site.

Recent Findings

Summerford

The four soil samples collected from Summerford (Table 3.2) were assessed as uncontaminated with respect to lead, zinc and copper. The maximum concentration of Pb, Zn and Cu in the soil were 418, 81 and 123 mg kg^{-1} respectively. These concentrations were below the ICRCL trigger threshold levels of 2000, 300 and 130 mg kg^{-1} respectively. However, the soils did contain marginally elevated concentrations of nickel at concentrations between 91 and 109 mg kg^{-1}, the maximum concentration exceeding the ICRCL value by 155%.

Chromium was elevated above the ICRCL threshold values for residential developments with gardens (600 mg kg^{-1}) and industrial use and open space levels (1000 mg kg^{-1}). The maximum concentration recorded exceeded the 600 mg kg^{-1} threshold by 344%. Based on the preliminary chemical test data the Summerford site was assessed as contaminated with chromium and marginally contaminated with nickel.

Redding

The seven soil samples collected from Redding were found to be uncontaminated with respect to chromium, however, a number of samples were found to contain elevated concentrations of copper, nickel, lead, zinc.

The chromium levels in the seven samples were recorded at concentrations between 15 and 27 mg kg^{-1} which is comparable with natural soils. Four samples contained elevated lead to a maximum concentration of 5643 mg kg^{-1}. Six samples contained

elevated concentrations of copper at concentrations ranging between 337 and 7310 mg kg^{-1}. Five of the samples contained elevated nickel at concentrations between 121 and 206 mg kg^{-1} and zinc was recorded at an elevated concentration of 827 mg kg^{-1} in one sample. The maximum concentrations recorded, exceed the ICRCL Guidelines by 282%, 5523%, 194% and 175% for lead, copper, nickel and zinc respectively.

The preliminary laboratory test data indicates that the soils collected from Redding are contaminated with lead, copper, zinc and nickel. The results show greater variation between individual samples which indicates the spread of contamination at Redding is likely to be random and 'hot spots' of contamination appear to exist.

Site Selection

Based on the historical site information, contamination levels determined by previous and preliminary site investigation works and the identification of suitable tree species growing at each site. The sites of Summerford and Redding were chosen as the study sites for the 'field investigation'.

A total of eight trees comprising three birch trees (*Betula pendula*) at Summerford and two birch, two willow (*Salix caprea L.*) and one sycamore (*Acer pseudoplatanus*) at Redding were studied over a two year period (March 1994 to March 1996). A further two trees comprising one willow (*Salix caprea L.*) and one birch (*Betula pendula*) were selected within area H4 at Redding. These trees were sampled and analysed during the first year only.

3.4 METAL AVAILABILITY

3.4.1 Introduction

The importance of metal and nutrient availability in soils has already been discussed in Chapter 1. The availability of metals in soils has a direct influence on the concentrations of metal taken up by plant roots. Soluble plant nutrients present in the soil solution are regarded as available and more likely to be taken up by plant roots compared to nutrients fixed within the soil matrix. In contaminated soils the total metal content of a soil is as important as the availability of the metals. In relation to plant uptake and toxicity symptoms, the effectiveness of cleaning up contaminated soils using vegetation (phytoremediation) will be dependent on metal availability in soil.

Plants also have the ability to alter the availability of metals in soils. Plants may secrete chemicals from the root surface, such as ligands and chelating agents, which can solubilise fixed metals in the soil (Wild, 1988). This process can increase the availability of metals and nutrients and may improve the potential of plants to remediate soils which contain fixed metals.

The soils collected from Redding and Summerford were assessed for metal availability by sequential extraction procedures to determine the availability of the heavy metals in the soil samples collected from each site. The laboratory procedures used are discussed.

3.4.2 Procedure

Shallow soil samples were collected from the rooting zone of the selected trees at both sites in accordance with the procedures documented in Chapter 2, Section 2.1.2 and were prepared in accordance with procedures documented in Section 2.1.4.

The prepared soil samples were analysed by non-sequential selective extraction procedures using calcium chloride, ammonium EDTA and oxalic acid. The soils were also analysed for residual metal content by hydrochloric/nitric acid digest. The acid digestion and selective extraction procedures were followed in accordance with documented procedures (Section 2.3.9 and 2.3.10) and the extracts were analysed for copper, zinc, chromium and lead by Flame Atomic Absorption in accordance with procedures documented in Section 2.5.

3.4.3 Results

The laboratory test results of the selective extraction and residual metal determinations of the collected soils are given in Table 3.4. The sampling programme had made an initial allowance for the collection of soil samples during each site visit. Due to the preparation time required the collection and analysis of soil was not continued throughout the duration of the 'Field' experiment.

Table 3.4. Non-sequential Selective Extraction Results of Soils beneath Selected Trees

	0.05M Calcium chloride				Ammonium EDTA				Oxalic Acid				Aqua-regia (HCl/HNO_3)			
	Zn	Cu	Pb	Cr	Zn	Cu	Pb	Cr	Zn	Cu	Pb	Cr	Zn	Cu	Pb	Cr
Summerford																
Tree 1-(6cm)	11	<1	5	14	17	18	134	21	65.4	30.7	19.3	244	131	72	297	1530
Tree 1-(25cm)	6	<1	7	27	14	24	122	24	64.0	41.6	14.9	279	145	98	321	1490
Tree 2-(6cm)	11	<1	8	20	18	21	198	40	45.7	36.7	32.8	259	129	86	355	1730
Tree 2-(25cm)	8	<1	12	24	15	23	213	29	73.5	51.7	25.9	285	131	87	361	1660
Tree 3-(6cm)	27	<1	6	19	31	24	153	28	97.2	56.5	42.2	292	170	98	300	1700
Tree 3-(25cm)	10	<1	39	27	20	26	663	110	71.3	54.6	83.3	377	139	101	774	1670
Redding																
H4-1	1100	31300	15000	2	1110	50900	18600	2.5	1190	79100	1460	0.8	2580	46800	14900	7.5
217-1	22	2	<2	1	26	9	38	<2.5	30.8	33.4	11.8	0.0	66.1	89	66	28
Birch-217	33	5	59	1	30	32	516	<2.5	70.3	71.6	285	1.1	484	132	1070	29
Willow-217	54	<1	78	<1	85	19	980	3	125	31.6	62.0	0.3	295	80	1130	32
Sycamore (6cm)	1700	30	806	<1	1280	53	1840	<2.5	1065	127	503	0.0	2510	214	2150	37
Sycamore (25cm)	162	10	819	<1	74	34	1780	<2.5	154	80.5	383	0.5	418	140	2720	47

Note: All values are expressed as mg kg^{-1} of soil

3.4.4 Discussion

Summerford

The selective extraction of copper, zinc, lead and chromium from the six Summerford soil samples confirmed that more metal was removed with the increasing vigor of the selective extractant used. It should be noted that oxalic acid was a poor extractant for lead which was attributed to the acid oxalate forming an insoluble precipitate with lead.

Copper: Calcium chloride extracted between 0.0 and 0.50 mg kg^{-1} of copper, ammonium EDTA removed between 17.7 and 26.2 mg kg^{-1} and the oxalic acid removed between 30.7 and 56.5 mg kg^{-1} of copper. The residual copper content of the soil was between 72.1 and 101 mg kg^{-1} of soil.

The percentage of copper in sample (Tree 3, 25cm) removed by oxalic acid, ammonium EDTA and calcium chloride, expressed as a percentage of residual was 54.3%, 26% and 0.50% respectively.

Zinc: Calcium chloride extracted between 5.6 and 27 mg kg^{-1} of zinc, ammonium EDTA removed between 14.4 and 30.6 mg kg^{-1} and oxalic acid removed between 45.7 and 97.2 mg kg^{-1}. The residual zinc level in the soil ranged between 129 and 170 mg kg^{-1}.

The percentage of the residual zinc in sample (Tree 3, 25cm) extracted by oxalic acid, ammonium EDTA and calcium chloride was 51%, 14% and 7.1% respectively.

Chromium: The calcium chloride extraction removed between 13.5 and 27.4 mg kg^{-1} of chromium, the ammonium EDTA removed between 20.8 and 110 mg kg^{-1} and the oxalic acid extraction removed between 244 and 377 mg kg^{-1}.

The residual chromium content of the soils ranged between 1490 and 1730 mg kg^{-1}.

The percentage of the residual chromium in sample (Tree 3, 6cm) extracted by oxalic acid, ammonium EDTA and calcium chloride was 17.2%, 1.6% and 1.1% respectively.

Lead: Calcium chloride extracted between 5.0 and 38.5 mg kg^{-1}, ammonium EDTA removed between 122 and 663 mg kg^{-1} and oxalic acid removed between 14.9 and 83.3 mg kg^{-1}. The residual lead levels in the soils varied between 297 and 774 mg kg^{-1}.

The percentage of the residual lead extracted from sample (Tree 2, 25cm) by oxalic acid, ammonium EDTA and calcium chloride was 7.2%, 59.1% and 3.2% respectively. As previously noted the results indicate the poor selective extraction of lead with oxalic acid.

Redding

On first inspection the chemical test results are more variable between individual soil samples collected.

Copper: Excluding the results from Site H4, the calcium chloride extraction removed between 0.4 and 29.9 mg kg^{-1} of copper, ammonium EDTA extracted between 9.0 and 53 mg kg^{-1} and the oxalic acid removed between 31.6 and 127 mg kg^{-1}. The residual copper concentration in the soils ranged from 80 and 214 mg kg^{-1}.

The percentage of residual copper extracted by oxalic acid, ammonium EDTA and calcium chloride varied from 38 to 59%, 10.1 to 25% and 0.5 to 14.0% respectively.

The selective extraction of the soil from site H4 with calcium chloride, ammonium EDTA and oxalic acid gave extractable concentrations of copper of 31300, 50900 and 79100 mg kg^{-1}. The residual copper concentration was 46800 mg kg^{-1} determined by acid digest.

It should be noted that ammonium EDTA and oxalic acid extracted a higher concentration of copper than the determined residual metal concentration. These results have been attributed to analytical error due to the highly contaminated nature of material. In this regard it has been assumed that ammonium EDTA and oxalic acid removed 100% of the residual metal, while calcium chloride removed 67% of the residual metal.

Zinc: The calcium chloride extraction removed between 21.8 and 1700 mg kg^{-1}, ammonium EDTA removed between 25.8 and 1280 mg kg^{-1} and oxalic acid removed between 30.8 and 1190 mg kg^{-1}. The residual metal content of the soils ranged from 66 and 2580 mg kg^{-1}.

The percentage range of residual zinc extracted by oxalic acid, ammonium EDTA and calcium chloride varied from 14.5 to 42.5%, 6.2 to 51% and 6.7 to 68% respectively.

Chromium: The calcium chloride extraction removed between 0.3 and 1.6 mg kg^{-1}, ammonium EDTA removed between 0.0 and 2.3 mg kg^{-1} and oxalic acid removed between 0.0 and 1.1 mg kg^{-1}. The residual chromium concentration of the soils ranged from 7.5 and 47.3 mg kg^{-1}.

The percentage of the residual chromium in the soil sample (Willow 217) extracted by oxalic acid, ammonium EDTA and calcium chloride was 1.0%, 8.7% and 0.9% respectively.

Lead: In general but excluding the results from sample H4 the calcium chloride extraction removed between 1.5 and 819 mg kg^{-1}, the ammonium

EDTA extracted between 38.2 and 1840 mg kg^{-1} and oxalic acid removed between 11.8 and 503 mg kg^{-1}. The residual lead level in the soil samples ranged from 65.9 and 2720 mg kg^{-1}.

The percentage of residual lead in the soil sample (sycamore 6cm) removed by oxalic acid, ammonium EDTA and calcium chloride was 23.4%, 85.8% and 37.6% respectively.

The selective extraction of the soil sample (H4) by calcium chloride, ammonium EDTA and oxalic acid removed 15000, 18600 and 1460 mg kg^{-1} of lead respectively. The residual lead concentration was 14900 mg kg^{-1}. In this regard the calcium chloride and ammonium EDTA removed 100% of the residual lead.

The theory of selective extraction procedures has been discussed in the Chapter 1. Non-sequential selective extraction or sequential selective extraction procedures are analytical tools which help to predict the availability of metals present in the soil to plants. When considering or studying the uptake of heavy metal by plants it is important to assess the availability of those metal in order to understand possible variations in uptake between different metals in a soil.

The extractants used were calcium chloride, ammonium EDTA and oxalic acid. Calcium chloride is a recognised extractant of the soil solution and exchangeable fraction in the soil. Ammonium EDTA is used to extract the exchangeable fraction plus the organically bound fraction in the soil. Oxalic acid removes the exchangeable, organically bound and the hydrous oxide bound fraction in the soil. By using such a series of extracting agents different 'pools' of soil metal can be extracted from the soil. It should be noted that oxalic acid was found to be a poor extractant of lead, this was thought to be due to the oxalic acid forming an insoluble precipitate with lead.

The selective extraction of the soils from Summerford indicate that the majority of the total residual metal in the soil is unavailable for plant uptake. Approximately 0.5% to 7.1% of residual copper, chromium, zinc and lead was removed by the acetic acid. It is this fraction which is readily available for plant uptake. The organically bound and oxide bound pools of metal in general do not exceed 50% of the total residual metal for the four metals analysed. The soil at Summerford is contaminated by chromium, however, the selective extraction results indicate the majority of the chromium is unavailable for plant uptake. The studies also indicate that higher concentrations of copper, chromium and lead were extracted from the deeper soil horizons, however, zinc concentrations were higher in the surface soils. This indicates the possible accumulation of zinc in the surface layers of the soil, possibly associated with soil organic matter. No such association was noted for copper, lead and chromium.

The total metal concentrations in the soils from Redding were much more variable between different samples. The percentage of available metal also tended to be higher and more variable between samples. Soil sample H4 contained highly elevated concentrations of copper and lead and moderately elevated levels of zinc. The selective extraction of the material confirmed that a high percentage (> 95%) of the total metal was available for plant uptake.

On the basis of the selective extraction of the soils from both the study sites it would appear that other than chromium the concentrations of metals likely to be available for uptake by vegetation are higher at Redding than at Summerford. The field experiment has been designed to determine the uptake of heavy metals from such sites and these results will be discussed later.

3.5 SOIL pH AND LOSS ON IGNITION (LOI)

3.5.1 Introduction

Loss on Ignition and pH values recorded for both Summerford and Redding soils are included for information. Both are important soil components which as previously discussed can have a significant influence on metal availability in soils. Soil pH can directly affect the solubility of metal complexes in the soil (generally metals become more soluble in acidic soils). Organic matter can chelate and fix heavy metals which can influence the availability of metals in the soil (Wild, 1988).

3.5.2 Procedure

Loss on ignition and soil pH (calcium chloride) were measured in accordance with the procedures documented in Chapter 2, Sections 2.3.1 and 2.3.3. The soil pH and loss on ignition results are included in Table 3.5.

3.5.3 Results

Table 3.5. Soil pH and Loss on Ignition

Sample Reference	Soil pH	% Loss on Ignition
Summerford		
Tree 1 (6cm)	7.1	23.3
Tree 1 (25cm)	7.4	13.7
Tree 2 (6cm)	6.7	26.8
Tree 2 (25cm)	7.5	12.9
Tree 3 (6cm)	6.4	24.4
Tree 3 (25cm)	7.4	13.9
Redding		
Site 217 back	4.9	18.1
Birch 217 front	5.2	15.6
Willow 217 front	7.2	14.6
Sycamore (6cm)	6.6	18.9
Sycamore (25cm)	7.2	11.5
Site H4		55.0

3.5.4 Discussion

The pH of the soils collected from Summerford vary between 6.4 and 7.4. and indicate the soils are marginally acid to marginally alkali. The pH of the soils collected from Redding ranged between 4.9 and 7.2 indicating some of the soils are slightly more acidic than the Summerford soils. On the basis of these pH determinations, heavy metals in the Redding soils are likely to more available for plant uptake.

Loss on Ignition values for Summerford soils ranged between 12.9 and 26.8 %. Soil samples collected from 25 cm depth contained approximately 50% of the organic matter recorded in surface samples and indicates the possible accumulation of organic matter in the surface soil arising from leaf litter and vegetation debris. As previously noted soil zinc levels would appear to be associated with soil organic matter.

The organic matter content of the Redding soils varies between 11.5 and 55%. Excluding the LOI value for sample H4 the % LOI varies between 11.5 and 18.9 which is slightly less than observed in the Summerford soils. Sample H4 had the appearance of an organic absorbent material like sawdust. This material may have been used to mop up spills of metal salts and this may explain the high available metal contents and high organic matter content in the sample.

3.6 BIOMASS ANALYSIS

3.6.1 Introduction

The Field Studies comprised the monthly collection and analysis of biomass samples collected from the selected study trees. The selection of relevant sites and sample trees was discussed previously in this Chapter.

3.6.2 Procedure

The biomass samples were sampled in accordance with the procedures documented in Chapter 2, Section 2.2 and were prepared and analysed in accordance with procedures documented in Sections 2.4 and 2.5.

3.6.3 Results

The results of the 'Field Study' investigation have been tabulated in Tables 3.6 to 3.17 which have been reproduced in the Appendix. As a result of the large quantity of data generated it has not been possible to display the data in a single table. A summary of the results contained within each table are listed below:

Table 3.6 Moisture content of Biomass Samples (Summerford)
Table 3.7 Moisture contents of Biomass Samples (Redding)
Table 3.8 Zinc in Biomass Samples (Summerford)
Table 3.9 Copper in Biomass Samples (Summerford)
Table 3.10 Chromium in Biomass Samples(Summerford)
Table 3.11 Lead in Biomass Samples (Summerford)
Table 3.12 Zinc in Biomass Samples (Redding)
Table 3.13 Copper in Biomass Samples (Redding)
Table 3.14 Chromium in Biomass Samples (Redding)
Table 3.15 Lead in Biomass Samples (Redding)

Table 3.16 Zinc, Copper, Lead and Chromium in Root Samples (Summerford and Redding)

Table 3.17 Zinc, Copper, Chromium and Lead in Biomass Samples (Site H4, Redding)

The limits of quantification calculated in Chapter 2, Section 2.5.5 have been utilised in the assessment of the results obtained. The limits of quantification for zinc, copper, chromium and lead were 1, 5, 5 and 20 mg kg^{-1} respectively for the analytical procedure used. In addition the reproducibility of the analysis procedure has been assessed as ± 7, 3, 5 and 5 mg kg^{-1} for zinc, copper, lead and chromium with respect to the analysis of the purchased Standard Reference Material.

Results have not been included for core samples for two reasons: the metal concentrations in the core samples were similar to concentrations recorded in wood samples and core samples were only collected during the sampling period March 1994 to October 1994.

The tabulated results (Tables 3.6 to 3.17) for the metal content of all biomass samples are included in Appendix 1. Some of the results have been expressed in graphical form in the following discussion.

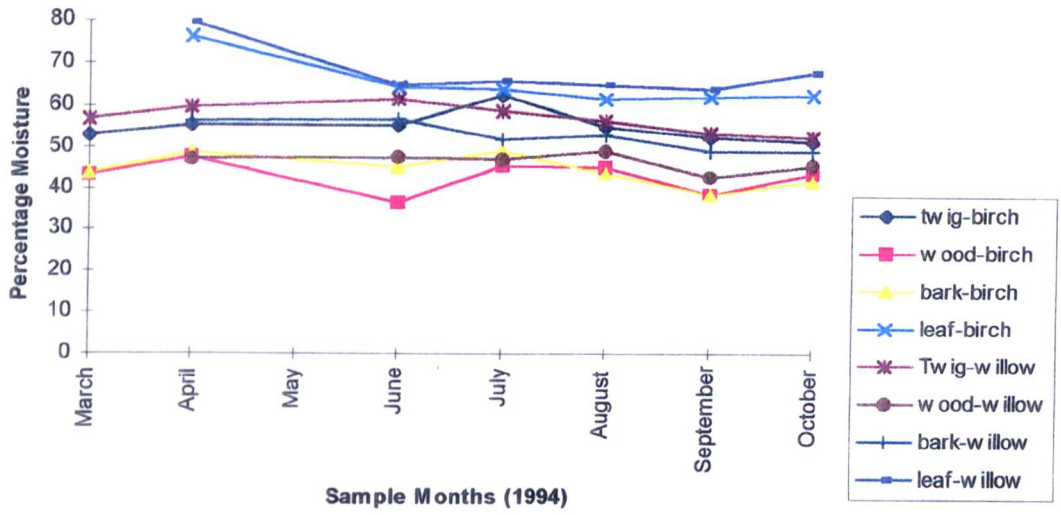
3.6.4 Discussion

It is important to consider moisture content before examining metal content in more detail because all metal contents are expressed on an oven dry basis. Therefore metal concentrations in fresh tissue will be influenced by the moisture content at the time of sampling.

Moisture Content

The moisture contents of the biomass samples collected at Summerford and Redding are tabulated in Table 3.6 and Table 3.7 respectively. The moisture contents of wood, bark, leaf and twigs Summerford birch trees and Redding willow trees have been presented in Figure 3.14 'Moisture Content of Birch (Summerford) and Willow (Redding)'.

Figure 3.14 Moisture Content of Birch (Summerford) and Willow (Redding)



Summerford

The moisture contents of the twig samples fluctuate between 40.4 and 62.5% during the season (29 March to 18 October 1994). The trend shows an early season increase in moisture content peaking in June/July for the 14 ft samples, the moisture content for 4 ft samples peaks earlier in the season. The 14 ft samples collected from each tree tended to have a higher moisture content than the 4 ft samples.

The moisture content of bark samples displayed similar seasonal trends as to those observed in twigs. The moisture content of bark was recorded at between 12.4 and 63.1% in the Summerford samples. Generally there was a slight increase in the

moisture content in the period March to June, followed by a slight fall towards the end of the season. An unusual dip in moisture content in September may have occurred due to the slightly longer storage of vegetation in the cold room prior to analysis.

The moisture content of the wood samples varied between 17.4 and 56.1%. The fluctuations in moisture content of wood was similar to the fluctuations noted for the bark samples described above. The wood samples also show an unusual dip in moisture content in September.

The moisture content of leaf samples from 4 ft and 14 ft show a similar trend for each birch tree. Throughout the sampling period the moisture contents of the leaves varied between 57.5 and 73.6 %. The differences between leaf samples taken from different heights was minimal. Generally the moisture content of the birch leaves was highest at the beginning of the season, falling in the first month, remaining fairly constant throughout the season before increasing slightly in October.

Redding

The moisture content of the willow twigs fluctuated between 27.8 and 61.1%, birch twig moisture contents fluctuated between 47.3 and 53% and the sycamore twig moisture contents fluctuated between 49.2 and 55.1%. The moisture content of the twig samples from the five trees show a similar trend through the season, an increase in moisture content to mid season before decreasing into autumn. Although the willow twigs tended to have a higher moisture contents than birch or sycamore twigs.

The moisture content of the wood samples at Redding varied between 29.1 and 51.9% in birch, 33.6 and 50.2% in willow and 42.7 and 50.2% in sycamore. The moisture content in all species generally decreased from spring to summer before increasing slightly in autumn.

The moisture content of bark samples fluctuated between 32.2 and 58.3 % in birch, between 36.8 and 58% in willow and between 44.6 and 52.7% in sycamore. The results generally indicate that the moisture content of the willow bark is generally higher than that of the birch or sycamore. The moisture content of all bark samples decreased in the period spring to autumn.

The moisture content of the leaf samples collected at Redding fluctuated between 59.1 and 78.2 % in birch, between 62.4 and 79.4% in willow and between 66.9 and 74.7% in sycamore leaves. The moisture content of sycamore leaves was generally higher. Moisture contents in the leaves of all species showed a significant early season decline followed by a marginal decrease into autumn.

In summary the moisture contents of the biomass samples were in the order of 50% ± 10%. This means that the metal concentrations measured and expressed as oven dry weight will be twice the concentration in living tissue when amended for moisture content. The metal concentrations discussed are expressed on an oven dry basis and have not been amended.

The fluctuations in moisture contents could be attributed to external atmospheric conditions or growth factors in the studied trees. This is highlighted in the leaves where the high early season moisture contents could be attributed to early season growth and development or conversely the drop in moisture content could be due to the increased development of leaf structure (lignin and carbohydrate) during the growing season. The recorded moisture contents could also be due to the atmospheric conditions prior to sampling.

Metal Contents

The measured metal contents of the biomass samples collected during the course of the 'field' experiment at Summerford and Redding are reproduced in Tables 3.8 to 3.17 which are included in Appendix 2. Given the large quantity of data the results

have been summarised in Tables 3.18 and 3.19 for Summerford and Redding respectively.

Table 3.18. Summary of Metal Content of Biomass (Summerford)

SITE	TREE REF.	SAMPLE	NO OF ANALYSIS	CONCENTRATION RANGE			
				ZINC	COPPER	CHROMIUM	LEAD
SUMMERFORD	BIRCH TREE 1	FINE ROOTS	7	14-52	5-26	18-157	<20-51
		LARGE ROOTS	6	13-45	<5-9	8-13.4	<20
		4 FT TWIGS	18	56-111	<5-14	<5	<20
		4 FT WOOD	11	17-37	<5-7	<5	<20
		4 FT BARK	11	68-100	6-11	<5	<20
		4 FT LEAF	13	87-294	<5-16	<5	<20
		14 FT TWIGS	18	78-110	<5-10	<5	<20
		14 FT WOOD	11	18-43	<5-23	<5-6	<20
		14 FT BARK	11	83-143	<5-10	<5	<20
		14 FT LEAF	13	86-239	<5-11	<5	<20
	BIRCH TREE 2	FINE ROOTS	7	21-46	7-31	32-151	<20-30
		LARGE ROOTS	6	29-51	<5-8	8-25	<20-20
		4 FT TWIGS	18	90-134	<5-11	<5	<20
		4 FT WOOD	11	27-50	<5-5	<5	<20
		4 FT BARK	11	100-179	<5-9	<5	<20
		4 FT LEAF	13	74-248	<5-12	<5	<20
		14 FT TWIGS	18	82-148	<5-60	<5	<20
		14 FT WOOD	11	18-59	<5-6	<5	<20
		14 FT BARK	11	109-177	<5-9	<5	<20
		14 FT LEAF	13	67-144	<5-11	<5	<20
	BIRCH TREE 3	FINE ROOTS	6	37-58	<5-12	11-66	<20-30
		LARGE ROOTS	7	22-101	<5-10	9-425	<20-85
		4 FT TWIGS	18	9-137	<5-8	<5	<20
		4 FT WOOD	11	30-59	<5-5	<5	<20
		4 FT BARK	11	106-145	<5-8	<5	<20
		4 FT LEAF	12	63-245	<5-7	<5	20
		14 FT TWIGS	18	101-142	<5-8	<5	<20
		14 FT WOOD	11	26-71	<5-5	<5	<20
		14 FT BARK	11	119-178	<5-8	<5	<20
		14 FT LEAF	13	69-215	<5-10	<5	<20

All values are mg/kg oven dry weight

Table 3.19. Summary of Metal Content of Biomass (Redding)

SITE	TREE REF.	SAMPLE	NO OF ANALYSIS	CONCENTRATION RANGE			
				ZINC	COPPER	CHROMIUM	LEAD
REDDING	BIRCH FRONT	FINE ROOTS	7	100-147	7-20	<5-13	98-307
		LARGE ROOTS	6	66-138	<5-11	<5	<20-68
		TWIG	18	148-244	<5-8	<5	<20-37
		WOOD	11	36-81	<5	<5	<20-26
		BARK	11	174-218	<5-7	<5	<20-41
		LEAF	13	56-269	<5-13	<5	<20
	BIRCH BACK	FINE ROOTS	7	66-152	8-16	<5-14	39-355
		LARGE ROOTS	6	88-151	<5-104	<5	21-767
		TWIG	17	165-237	5-12	<5	<20-41
		WOOD	10	44-106	<5-6	<5-7	<20-25
		BARK	10	187-275	<5-26	<5	<20-62
		LEAF	12	83-588	<5-15	<5	<20
	BIRCH H4	FINE ROOTS	5	76-1120	120-7290	<5-8	37-5050
		LARGE ROOTS	5	64-469	32-1700	<5	<20-725
		TWIG	6	202-244	10-11	<5	<20-20
		WOOD	6	33-79	5-6	<5-6	<20-29
		BARK	6	240-281	7-11	<5	<20-25
		LEAF	5	219-425	5-15	<5-19	<20
	WILLOW FRONT	FINE ROOTS	7	82-134	5-80	<5-10	82-573
		LARGE ROOTS	6	76-122	6-12	<5	41-213
		TWIG	18	94-167	10-16	<5	<20-32
		WOOD	11	15-52	<5-15	<5	<20
		BARK	11	115-221	<5-9	<5	<20-23
		LEAF	13	59-133	6-19	<5	<20
	WILLOW BACK	FINE ROOTS	7	123-185	8-41	<5-88	54-128
		LARGE ROOTS	6	99-161	<5-25	<5-5	<20-312
		TWIG	18	141-260	8-51	<5	<20-22
		WOOD	11	23-71	8-16	<5-7	<20
		BARK	11	204-344	<5-7	<5	<20-22
		LEAF	13	103-245	6-14	<5-5	<20

Table 3.19. continued

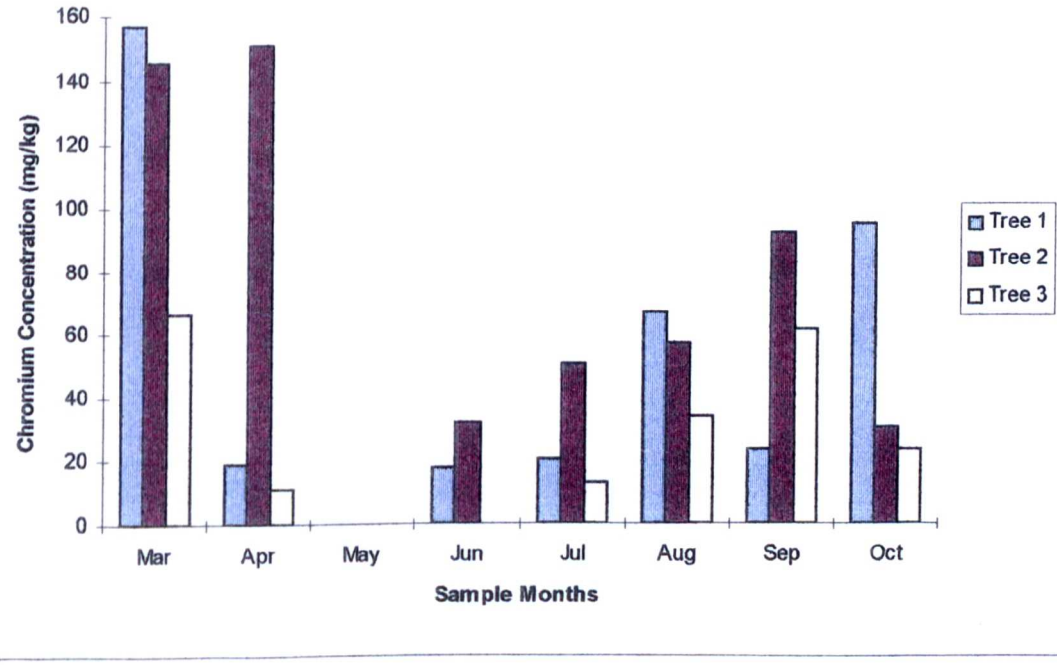
SITE	TREE REF.	SAMPLE	NO OF ANALYSIS	CONCENTRATION RANGE			
				ZINC	COPPER	CHROMIUM	LEAD
	WILLOW H4	FINE ROOTS	5	75-327	445-1190	<5	54-937
		LARGE ROOTS	5	44-125	86-249	<5	<20-232
		TWIG	6	159-182	13-16	<5	<20
		WOOD	6	41-67	<5-21	<5-6	<20-29
		BARK	6	247-333	<5-12	<5	<20
		LEAF	5	159-223	7-9	<5-12	<20
	SYCAMORE	FINE ROOTS	7	48-916	12-61	<5	84-2920
		LARGE ROOTS	6	20-141	5-13	<5	21-224
		TWIG	18	6-28	<5-6	<5	<20-29
		WOOD	11	4-22	<5	<5	<20
		BARK	11	17-36	<5-7	<5	<20-26
		LEAF	13	21-57	<5-9	<5	<20

When the limits of quantification are applied to the chromium and lead results a number of the laboratory test results for chromium and lead are below the limit of quantification of the analytical procedure used. In this regard results which fall below the level of quantification have been expressed as less than quantification limit. It should be noted that the purpose of the experiment was to find if trees growing on contaminated sites accumulated high levels of heavy metals and in this regard a result less than the quantification limit indicates that no significant uptake of metal has occurred.

Chromium

Chromium was not accumulated by any of the study trees. The highest concentrations of chromium were recorded in the roots of birch growing on chromium contaminated spoil and these results may have been influenced by adhering soil, although every effort was made to remove this soil by washing. The recorded chromium concentrations in fine birch roots growing at Summerford are presented in Figure 3.15.

Figure 3.15 Chromium in Fine Roots (Summerford)

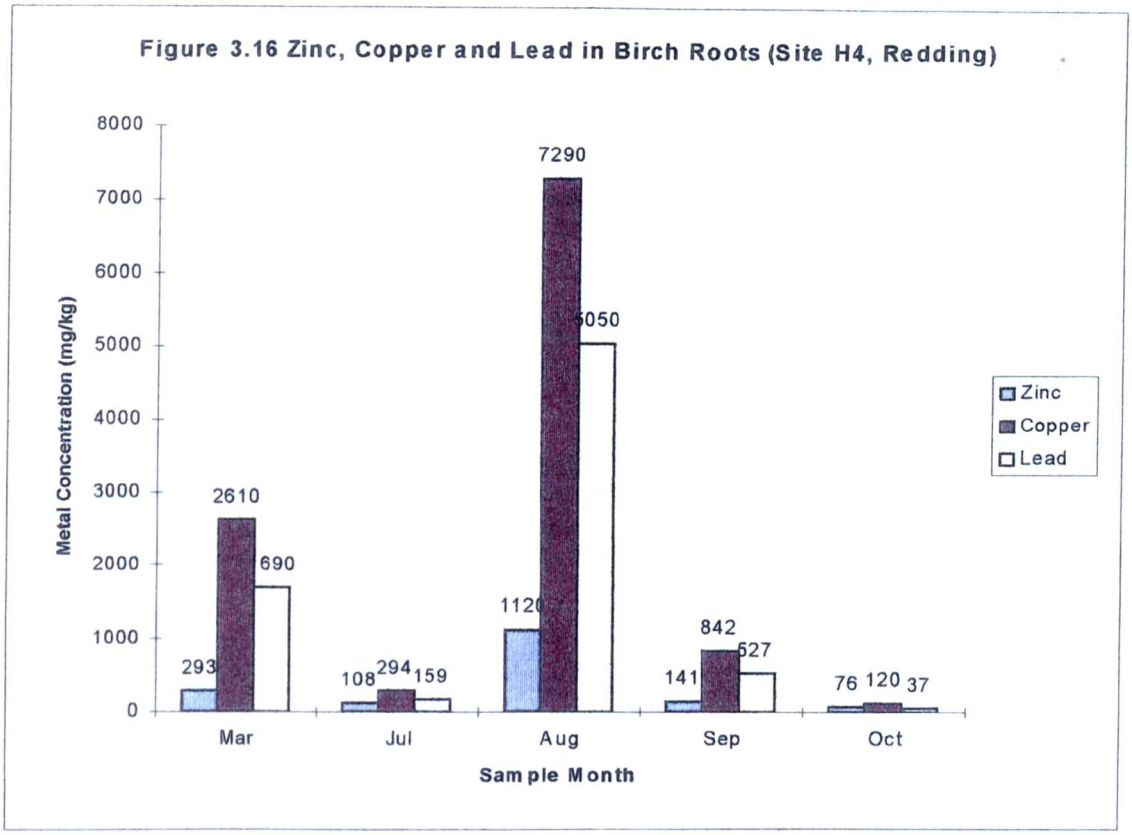


The levels of chromium recorded in the various birch roots (Summerford) vary between sampling 20 and 160 mg kg^{-1}. Studies by Moral et al. (1994) reported that chromium was fixed in the roots and was therefore not transported to above ground components.

No difference in the uptake of chromium was noted in the selected trees between the two study sites even although only the Summerford site was contaminated with chromium. In this regard the study trees, especially birch do not appear to take up or accumulate chromium in above ground biomass.

Lead

Lead was recorded to be present to the highest concentrations within the fine roots of the study trees. As with copper the highest concentrations were in the roots of trees growing in spoil contaminated with high concentrations of available lead. In the above ground biomass, lead tended to be more concentrated in the bark and less in the leaves. This may indicate the very slow accumulation of lead over a number of years due to the atmospheric deposition and binding to the external surfaces of twigs and stem. The concentration of lead measured in the roots of birch at Redding are shown in Figure 3.16 'Zinc, Copper and Lead in Birch Roots (Site H4, Redding).



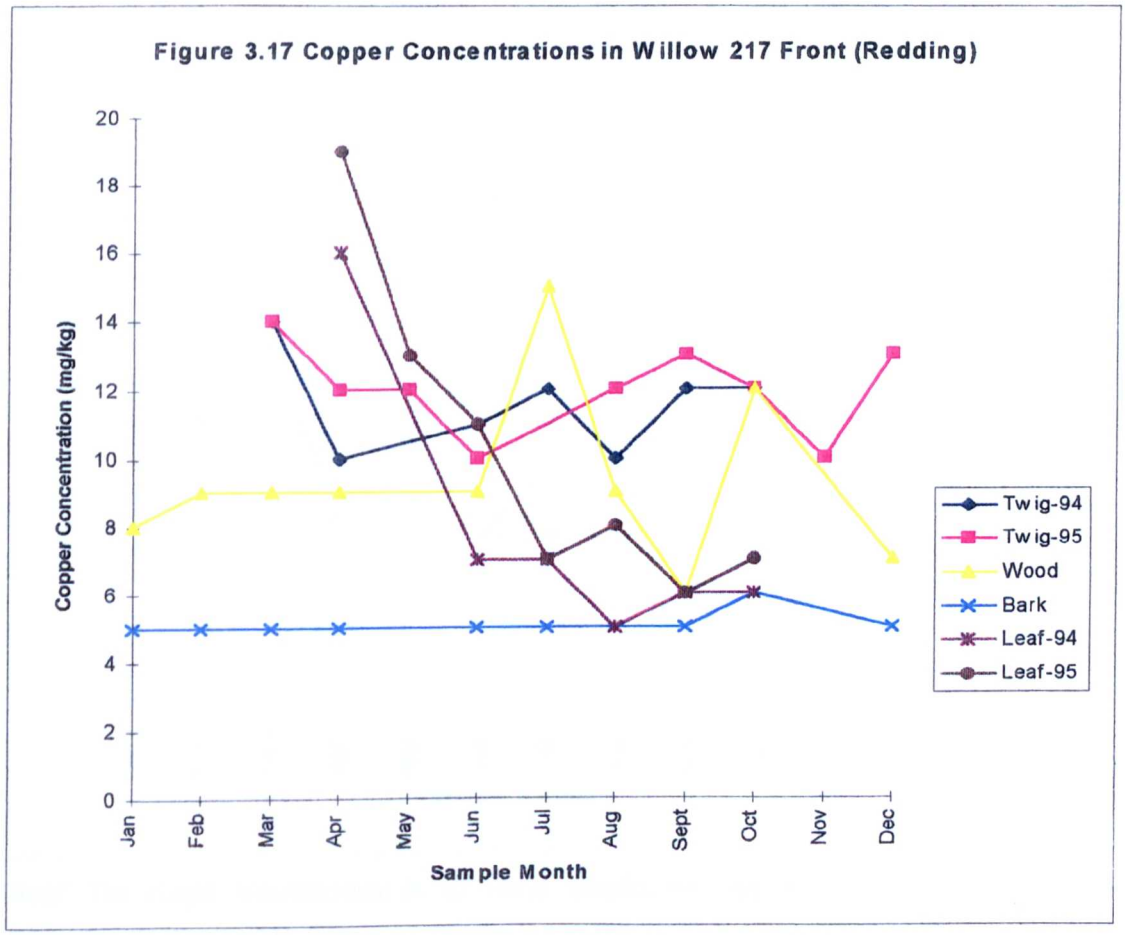
For comparison the concentrations of copper and zinc recorded in the roots of birch tree H4 have been included for comparison. The accumulation of the three metals in tree roots was Cu > Pb > Zn, to maximum concentrations of 7290, 5050 and 1120 mg kg^{-1} respectively. Eltrop et al. (1991) reported zinc levels in the roots of birch (*Betula*) and willow (*Salix*) roots growing on mine tailings of 20969 and 8467 mg kg^{-1} respectively. Total lead concentrations in the mine spoil were higher but exchangeable levels were less.

Due to the relatively low levels of lead and chromium recorded within the 'field' samples no further discussion will be given on the fluctuations of these metals within different components during the season. It is worth noting that given the very low uptake of the metals it can be concluded that the study species did not accumulate lead or chromium under the experimental conditions studied and therefore phytoremediation of these metals on the study sites using birch, willow or sycamore would not appear to be a viable option. The results do indicate that lead and chromium may be strongly held in the roots of trees. This binding in the roots could remove available metal from the soil solution which could reduce the potential for copper and lead to leach from contaminated soils.

Copper

Copper was recorded at highest concentrations within the roots of the study trees and lower concentrations in the leaves, wood and twigs. However, unlike lead and chromium the concentrations recorded in wood, twigs and leaves were generally above the limit of quantification of 5 mg kg^{-1}. The reproducibility of the analysis was determined as ± 1.8 and 3.5 mg kg^{-1} in pine and olive leaves respectively.

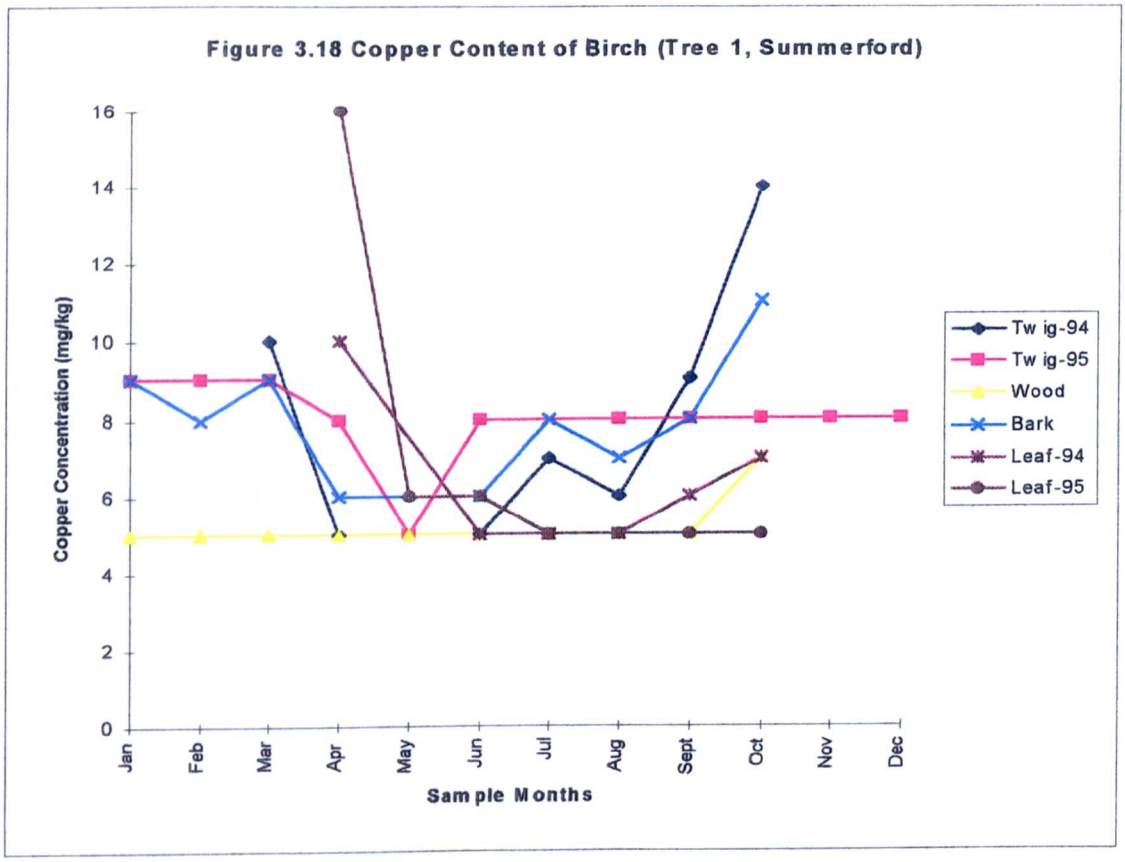
The fine root samples collected from birch growing within area H4 at Redding contained maximum copper concentrations of 7290 mg kg^{-1} oven dry weight (Figure 3.16). However in all the study trees no high accumulation of copper was noted in above ground components. The measured concentrations of copper within the study trees are presented in Figure 3.17 'Copper Concentrations in Willow 217 Front (Redding)' and Figure 3.18 'Copper Content of Birch (Tree 1, Summerford)'.



Note: The copper concentration in bark recorded in March, April, June, July, August and December were less than detection limit.

The distribution of copper within the different components of willow growing at Redding, in order of highest to lowest concentration is leaf, twig >wood > bark. Copper concentrations in leaves appear to fall during the season, however this may be due to an increase in dry matter content rather than the transportation of copper from the leaf (a dilution effect). This dilution effect was reported by Fromm et al. (1987) when copper and nickel concentrations were found to decrease with increasing growth. The concentrations in twigs appears to be more constant.

These results indicate that even in soils containing high concentrations of available copper, copper is preferentially bound and retained in the roots and is not transported above ground. This may also indicate the regulation of copper by the tree species studied. The results generally indicate that the regulated concentration of copper within trees is between 5 and 20 mg kg^{-1} dry weight.



Note: The copper concentration in all wood samples and leaf samples June'94, August'94, September'94, October'94, August'95, October'95 and November'95 were less than detection limit.

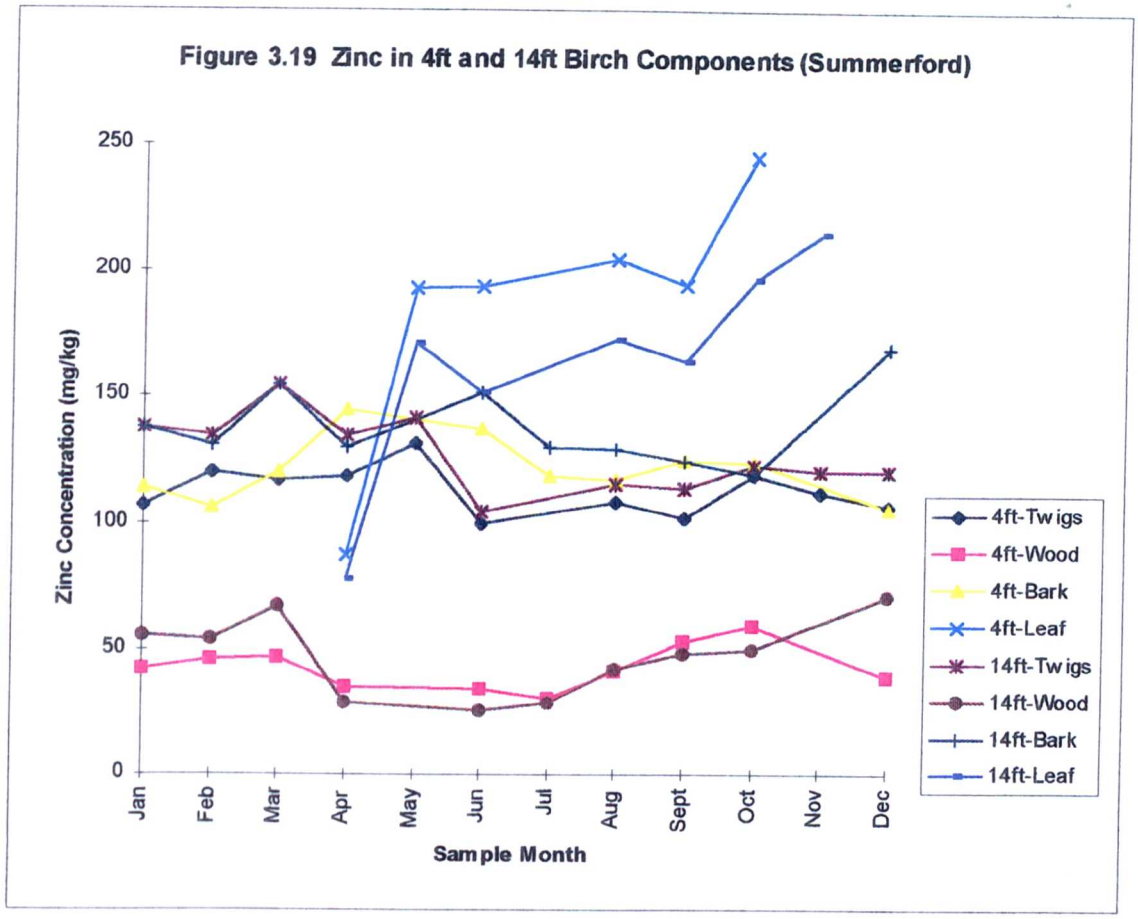
The copper concentrations in the components of birch tree 1 growing at Summerford are marginally lower than the concentrations recorded in willow growing at Redding. The distribution of copper within the different components of birch tree 1 growing at Summerford, in order of highest to lowest concentration is leaf, twig > bark > wood. The regulated concentration of copper is between 5 and 16 mg kg^{-1}.

Zinc

With respect to zinc the calculated limit of quantification was assessed as 1 mg kg^{-1} and the variation in the analysis of the olive leaf standard reference material was ± 6.8 mg kg^{-1}.

Zinc was the only studied metal which was recorded within foliage at concentrations comparable with levels detected in the soil. The concentrations in above ground biomass was higher than the zinc concentrations detected in the roots, apart from the trees growing in area H4 which contained high concentrations of available zinc in the soil.

The distribution of zinc in different above ground components of birch are presented in Figure 3.19 'Zinc in 4 ft and 14 ft Birch Components (Summerford)'. The distribution of zinc between the different components in order of highest to lowest concentration is leaves > bark > twigs > wood. No significant difference is noted between samples collected from different heights. The results also show the seasonal accumulation of zinc in leaves.



The zinc concentrations recorded within foliage, twigs, wood and bark of the eight study trees are presented in Figure 3.20 to Figure 3.23 respectively.

Figure 3.20 'Zinc Levels in Foliage of Birch, Willow and Sycamore' shows the seasonal fluctuation of zinc in leaves. The most significant seasonal accumulation of zinc is in birch tree 217 back (Redding) which accumulates twice the level of zinc (500 mg kg^{-1}) in foliage compared with the other trees. The order of zinc accumulation in foliage between different species was birch > willow > sycamore.

Figure 3.20 Zinc Levels in Foliage of Birch, Willow and Sycamore

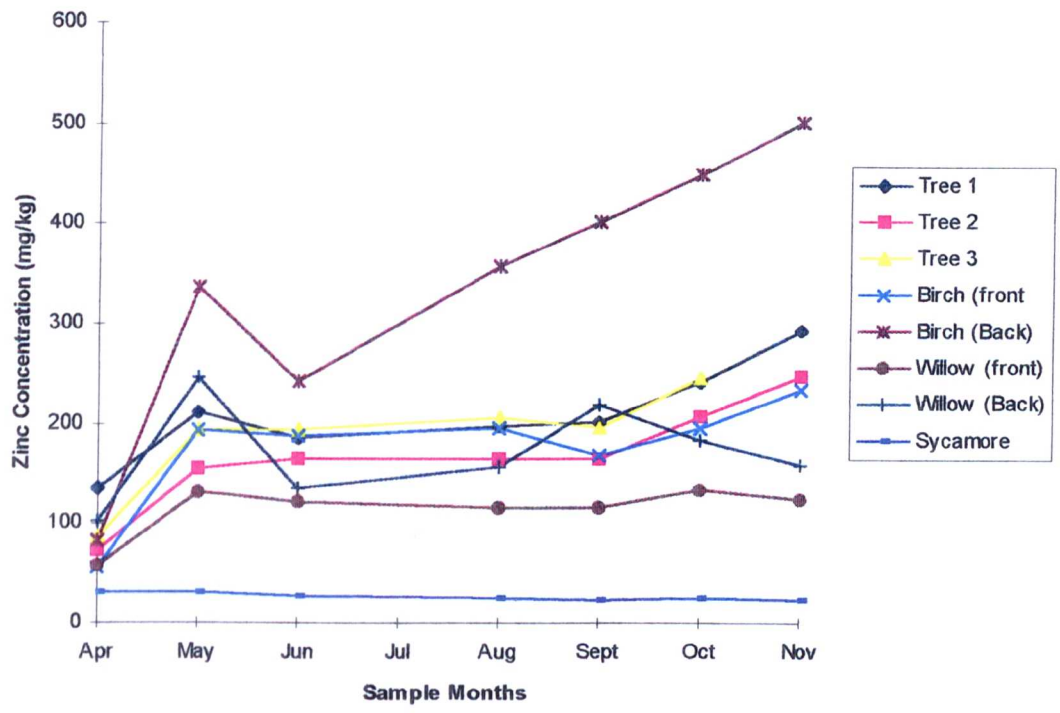


Figure 3.21 'Zinc Levels in Twigs of Birch, Willow and Sycamore' shows the recorded zinc concentrations in the twigs of the eight study trees. The accumulation of zinc in twigs was only marginally less than the accumulation in leaves. With respect to the species of tree, zinc accumulation in order to highest to lowest concentration was birch (Redding) > willow (Redding) > birch (Summerford) > sycamore. No seasonal trends or fluctuations were noted.

Figure 3.21 Zinc Levels in Twigs of Birch, Willow and Sycamore

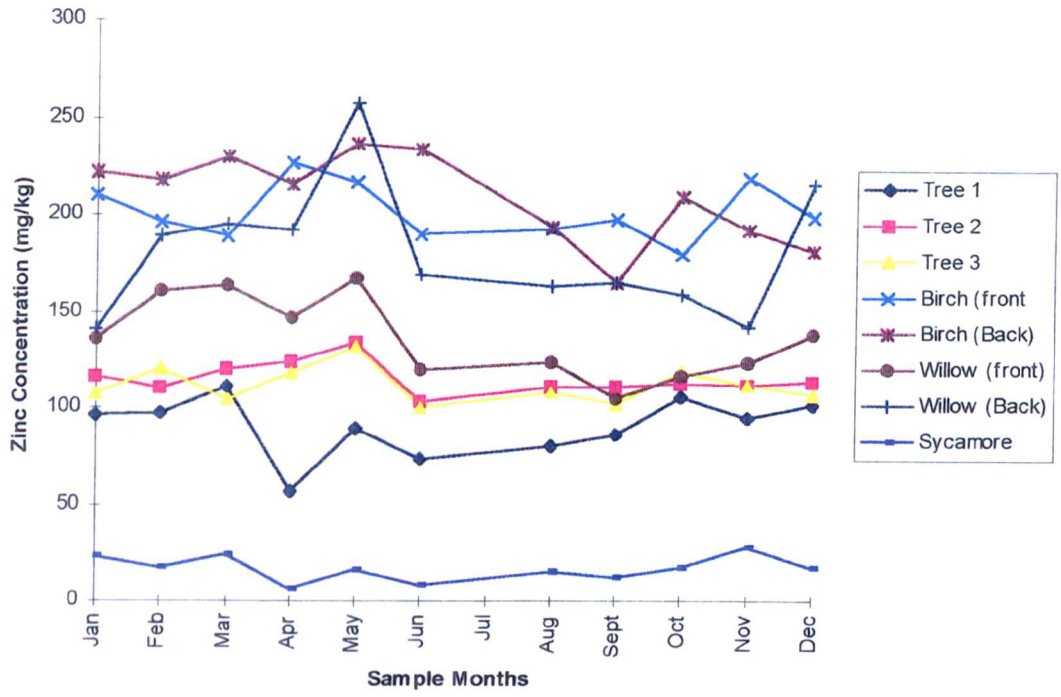


Figure 3.22 'Zinc Levels in Wood of Birch, Willow and Sycamore' shows the seasonal fluctuations of zinc in the wood of the eight study trees. The accumulation of zinc in wood of different species was similar to that observed in foliage samples, although the concentrations were less. The order of accumulation within the different species in order of highest to lowest concentration was birch > willow > sycamore. In wood the zinc concentrations display a trend of early season high, a mid season decrease and late season increase.

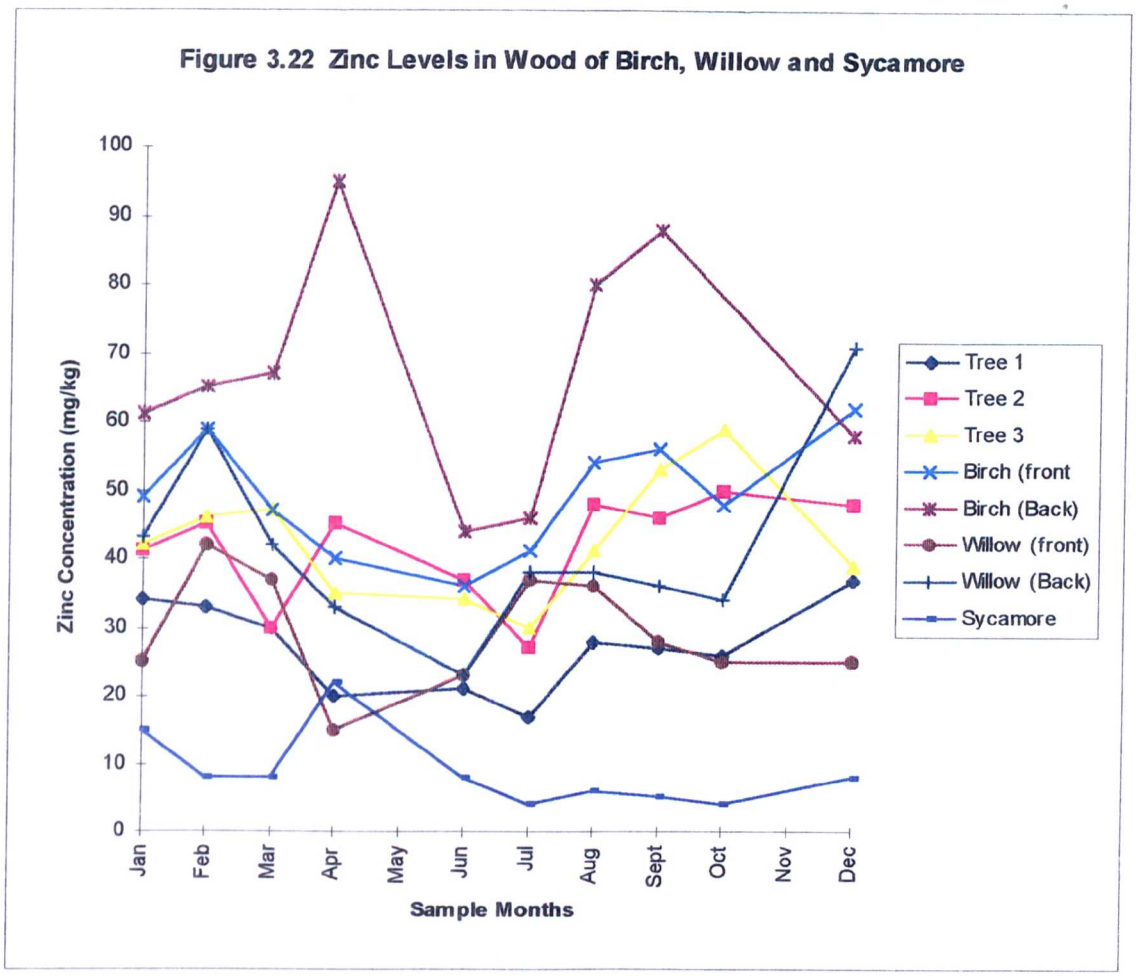
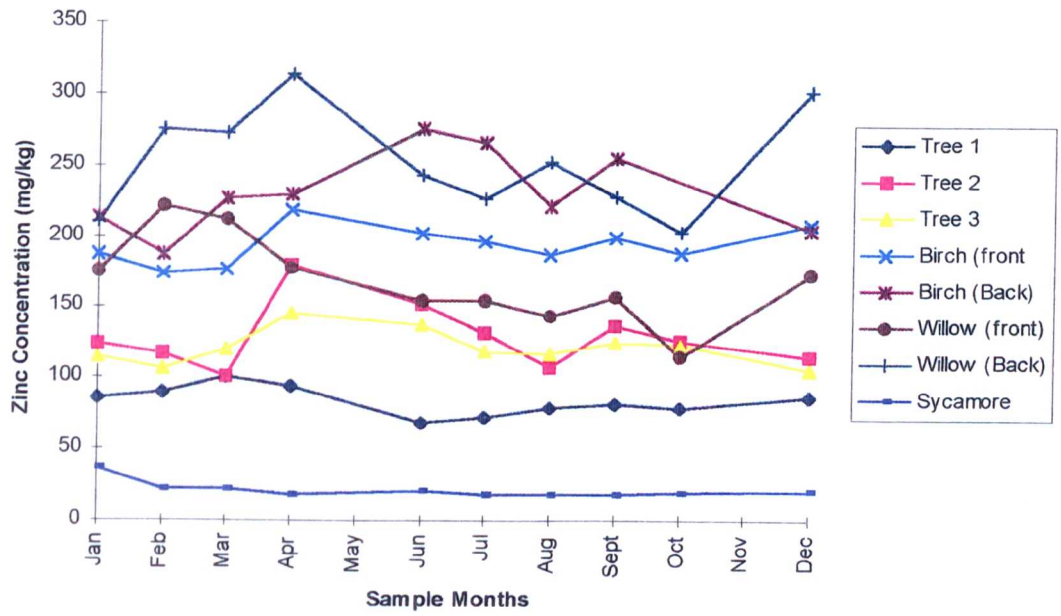


Figure 3.23 'Zinc Levels in Bark of Birch, Willow and Sycamore' shows the seasonal zinc levels recorded in the bark of the eight study trees. The order of zinc accumulation in bark between the different trees was in the order of highest to lowest concentration was birch (217 back) > willow (217 back) > birch (217 front) > willow (217 front) > birch (Summerford) > sycamore. In bark the zinc concentrations show a trend of an early season high, a mid season decrease and late season increase. Young (1971) reported a 4.6 : 1 ratio between metal levels in bark and wood. From the studies presented here a similar ratio exists for the zinc levels recorded in the bark and wood of the study trees.

Figure 3.23 Zinc Levels in Bark of Birch, Willow and Sycamore



In summary the distribution of zinc within the study trees in order of highest accumulation to lowest appears to be leaves > bark > twig > roots > wood. These findings are similar to those reported by Hogan and Morrison (1988) whereby the highest concentrations of metals were found in foliage and bark samples and the lowest were in stemwood.

As discussed during the season (January to December) the zinc concentrations increase in leaves while in bark and wood the zinc concentrations show an early season high, a mid season decrease and late season increase. No obvious trends were noted in twig samples. These trends may indicate the transfer of zinc within the plant to leaves for growth during summer and the recovery of zinc from leaves to bark and wood as senescence approaches. These findings are similar to those by Mejnartowicz (1986) who reported the accumulation of copper and zinc in shoots during the winter which was subsequently transferred to the leaves during foliation.

The levels of zinc, copper and lead recorded in the foliage of the study trees growing on contaminated sites are slightly lower than the concentrations recorded in vegetation growing on smelter sites. Hogan and Wotton (1984) measured the concentrations of zinc, copper and lead in the foliage of four tree species (Jack pine, Black Spruce, Alder and Labrador Lea) and recorded the following range of concentrations; 53-506 mg kg^{-1} zinc, 2-38 mg kg^{-1} copper and 5-62 mg kg^{-1} lead. Rachwal (1983) measured maximum zinc, copper and lead levels in poplar species (*P. marilandica*) to 211, 506 and 124 mg kg^{-1} respectively. It should be noted that smelter sites often emit high concentrations of atmospheric contamination which may be the cause of the higher metal concentrations recorded in foliage.

Further discussions of the results will be given in Chapter 6.

CHAPTER 4
POT EXPERIMENT

4.1 INTRODUCTION

The 'pot experiment' refers to a green house experiment where selected tree seedlings were planted in artificially contaminated soil. The experiment was designed to study the impact and uptake of heavy metals on four selected tree species growing in uniformly contaminated soil. A large greenhouse was used to create a confined and controlled environment for the cultivation of the trees.

Related experimental studies on established trees growing in heavy metal contaminated soils were discussed in Chapter 3 and nutrient solution studies (Chapter 5) have been undertaken in conjunction with this experiment in an attempt to gain further insight into the relationship between metal uptake by plants and metal concentrations in the soil and soil solution.

Observing and analysing trees and plants which have colonised heavy metal polluted soil/waste is one method of studying metal uptake by vegetation. However, this type of study presents a number of problems; the polluted soil/waste tends to be of a non-uniform composition which makes the reproducibility of results difficult and large analytical differences can be due to variations in the soil metal concentration rather than plant factors. In some instances root distribution may be confined to the less polluted areas of the soil and environmental factors such as drought and rainfall can have an effect especially from year to year.

The pot experiment was designed to allow the study of plants growing in soil contaminated with known concentrations of heavy metal. The experiment was devised to allow the replication of identical soil and metal treatments. Under these conditions the trees would experience similar conditions of moisture, temperature and light. Therefore any variation in metal uptake would be a factor of the tree species. This type of experiment allows the observation of changes in the trees caused by the metal treatments. The main changes likely to be observed will be to the growth, tolerance, survival and nutrient deficiency symptoms of the seedlings.

4.1.1 Methodology

Details of the experimental design and experimental setup are given in Chapter 2, Section 2.6 titled 'Pot Experiment'.

4.2 DETERMINATION OF SOIL PROPERTIES

4.2.1 Physical and Chemical Soil Properties of Original Soil

4.2.1.1. Introduction

The physical and chemical properties of a soil are individual to that soil. When studying the behaviour of heavy metals added to a soil the physical and chemical properties of the soil are very important and ultimately influence the behaviour of heavy metals and nutrients within that soil.

Soil pH can directly influence the cation exchange capacity of a soil which in turn can influence the solubility of inorganic complexes formed in the soil. In soils with high cation exchange capacity, heavy metals in the soil solution will be attracted to and held on exchange sites. When the cation exchange capacity of the soil is reduced by changes in soil pH some of the held heavy metals may be released into the soil solution. Heavy metals also become more soluble under acidic soil conditions. In this regard heavy metal availability in the soil is influenced by changes in soil pH.

Soil texture is linked to the respective particle size distribution and percentage of sand, silt and clay fractions in the soil. The experimentally determined particle size distribution of a soil can be used to classify each soil. The silicate clay and organic matter in the soil have charged surfaces and ionised groups which give a soil its cation exchange capacity. As discussed above the soil pH can influence the degree of negative charge and therefore the cation exchange capacity of a soil.

These negative charges on the clay and soil organic matter provide sites at which cations in the soil can be held by ion-exchange. A measure of the total negative charge and the percentage of ion exchange sites in a soil holding calcium, magnesium, sodium and potassium cations can be determined experimentally and is termed cation exchange capacity and base saturation.

4.2.1.2. Procedure

A sub-sample of the 'pot' soil was analysed in the laboratory for soil pH, particle size determination, cation exchange capacity and base saturation.

The procedures used for the above determinations are provided in Chapter 2, Analytical Methods, Section 2.3.

4.2.1.3. Results

The physical and chemical parameters of the soil used in the 'Pot' experiment are given in Table 4.1. It should be noted the chemical parameters were determined on air dried and sieved soil (< 2 mm).

Table 4.1. Physical and Chemical Soil Properties

PHYSICAL PARAMETERS	
Grit/stones > 2 mm	52.6%
Soil < 2 mm	47.4%
Coarse to medium sand	32.0%
Fine sand	29.6%
Silt	15.9%
Clay	15.1%
Organic Matter (loss on ignition)	7.4%
CHEMICAL PARAMETERS	
Soil pH (calcium chloride)	4.9
Soil pH (water)	5.3
Soil salinity	0.2 mS cm^{-1}
Cation Exchange Capacity	16.8 $cmol_c$ kg^{-1}
Base Saturation	47.0%

4.2.1.4 Discussion

From the physical determination of the 'pot' soil 52.6% of the soil comprises material > 2mm and 47.4% < 2mm. The % of material > 2mm is likely to be higher due to the addition of coarse grit to the soil to help drainage. In this regard the pot soil is likely to be free draining.

The particle size determinations recorded 61.6 % sand, 15.9% silt, 15.1 % clay and 7.4 % organic matter. When the percentages are compared with the textural classes of soil used by the United States Soil Survey (Childs, 1974) the soil falls within the classification of a sandy loam.

The soil pH has been assessed as 5.3 in water and 4.9 in calcium chloride which indicates that the soil is of slight to moderate acidity (Weier et al., 1982) when compared with 'typical' agricultural soils. It is understood the soil was recovered from former grassland and in this regards such a soil pH is not unexpected.

The soil salinity was assessed as 0.2 mS cm^{-1}, this value when compared with conductivity index issued by MAFF (1988) suggests that 'no growth restrictions should apply'.

The cation exchange capacity has been assessed as 16.8 $cmol_c$ kg^{-1} .The calculated value is lower than the CEC of a bentonite clay which can be in the order of 40 $cmol_c$ kg^{-1} (Adamcová, 1999). In addition the base saturation is 47% which indicates some leaching of exchangeable cations (Mg, Ca, Na and K) has occurred. This value corresponds with what would be expected given the measured pH of the soil.

4.2.2 Soil Salinity

4.2.2.1. Introduction

Soil salinity is normally a problem associated with arid climates or greenhouses where a combination of excessive irrigation combined with high water evaporation rates can increase the concentration of salts in the soil. When these salts reach significant concentrations the soil solution can have a high salinity which can cause water stress to intolerant species (Weier et al., 1982).

An assessment of soil salinity was made on the pot soils at periods of 10 and 26 months after the addition of the metal salts. The reason for measuring soil salinity was that a considerable weight of inorganic salt was added to each pot to create the desired metal concentration and it is likely that when the salts dissolve in soil solution a potential salinity problem may arise to the planted trees. Therefore any potential stress effects shown by the tree seedlings may be due to problems with salinity and water stress rather than metal toxicity. This is why it was considered necessary to determine the salinity of the soil in each metal treatment.

4.2.2.2. Procedure

The control and heavy metal contaminated soils from the 'pot experiment' were sampled in February 1995 and August 1996 (10 and 26 months after the addition of the metal salts to the soils). The salinity of the collected samples were determined by laboratory analysis in accordance with the procedure given in Chapter 2, Section 2.3.2.

4.2.2.3. Results

The conductivity values are given in Table 4.2., the soil metal treatments having been described earlier in Section 2.6.2 and Table 2.5.

Table 4.2. Salinity of Artificially Contaminated Soils

Soil Metal Treatment (mg/kg)	Salinity μS cm^{-1} (Electrical Conductivity)	
	1995	1996
500 zinc	1950	1750
3000 zinc	6320	8110
500 copper	1790	530
2000 copper	4200	2610
500 nickel	3270	3500
1000 nickel	4200	3900
300 cadmium	1350	1050
1000 cadmium	2860	2540
2000 lead	1600	610
2000 chromium	3520	3020
control	190	210
distilled water	0	0

4.2.2.4. Discussion

For guidance the salinity values obtained can be compared with conductivity index figures published by MAFF (1988). The figures are reproduced in Table 4.3 and give an indication of soil salinity values which are likely to be detrimental to the health of agricultural crops.

The conductivity values are measured in saturated calcium sulphate solution at 20 ^{0}C. As a result conductivity values measured in water have to be amended so that results

can be compared. In this regard the conductivity of saturated calcium sulphate is given as 1.96 Dc/m. The electrical conductivity values in Table 4.3 has been amended to express conductivity values in water.

Table 4.3 Table of Conductivity Index

Conductivity Index	**Electrical Conductivity Dc/m**	**Electrical Conductivity $\mu S\ cm^{-1}$**
0	0.00-0.30	0-300
1	0.31-0.50	310-500
2	0.51-0.70	510-700
3	0.71-0.80	710-800
4	0.81-0.90	810-900
5	0.91-1.10	910-1100
6	1.11-1.40	1110-1400
7	1.41-1.80	1410-1800
8	1.81-2.10	1810-2100
9	>2.10	>2100

MAFF suggest no growth restrictions should apply to agricultural crops (conductivity index 0-2), possible growth restrictions may be experienced in young plants (conductivity index 2-4) and severe crop damage is likely (conductivity index >4).

The results of the soil salinity assessment indicate that soil treatments of 3000 mg kg^{-1} zinc, 2000 mg kg^{-1} copper, 500 and 1000 mg kg^{-1} nickel, 1000 mg kg^{-1} cadmium and 2000 mg kg^{-1} chromium have salinity values in excess of 2100 $\mu S\ cm^{-1}$ and severe toxicity damage is likely. The treatments of 500 mg kg^{-1} copper, 300 mg kg^{-1} cadmium and 2000 mg kg^{-1} lead were the only treatments with measured conductivity values below or close to index 4. The survival rates of the tree seedlings will be discussed later in this chapter. Comparison of the salinity values with time generally indicates that the salinity of the treated soils have decreased marginally with time. This may be attributed to the uptake of salts by trees, complexing of salts in the soil or the leaching of metal salt from the soil.

4.2.3 Soil pH

4.2.3.1. Introduction

The pH of the topsoil used in the 'pot experiment' was assessed on a sample of soil collected at the onset of the experiment and the results were reported in Table 4.1. Sub-samples of the pot soils sampled in February 1995 and August 1996 were further analysed for soil pH to assess if the addition of individual metal salts was influencing soil pH. In addition the soil pH was assessed with regard to individual tree species to see if one of the study species could alter soil pH.

4.2.3.2. Procedure

The soil pH was measured in accordance with the procedure detailed in the Chapter 2, Section 2.3.1. It should be noted that 0.01M calcium chloride was used and the soil suspensions were filtered through No 1 filter papers prior to analysis. The pH of the soil was determined on the extracted solutions resulting from the selective extraction of the soils. The results of the soil pH determinations are reported in Table 4.4.

4.2.3.3. Results

Table 4.4. pH of 'Pot Soils'

Soil Treatment	Poplar		Pine		Willow		Alder	
	1995	1996	1995	1996	1995	1996	1995	1996
Control	4.9	4.5	4.9	4.9	4.9	4.4	4.9	4.3
500 Zn	4.7	4.3	4.7	4.2	4.7	4.2	4.7	4.4
3000 Zn	4.8	4.5	4.7	4.8	4.8	4.5	4.7	4.6
500 Cu	4.6	4.4	4.7	4.4	4.7	4.3	4.6	4.2
2000 Cu	4.5	4.4	4.5	4.2	4.4	4.2	4.5	4.2
500 Ni	4.6	4.2	4.6	4.3	4.6	4.4	4.6	4.2
1000 Ni	4.6	4.2	4.6	4.2	4.6	4.2	4.6	4.2
300 Cd	4.8	4.5	4.8	4.3	4.8	4.3	4.8	4.3
1000 Cd	4.8	4.2	4.9	4.4	4.7	4.5	4.9	4.4
2000 Pb	4.9	4.4	4.7	4.4	4.4	4.4	4.8	4.5
2000 Cr	6.1	6.1	6.1	6.5	6.0	6.3	6.1	6.3

4.2.3.4. Discussion

The initial pH of the soil used in the experiment was assessed at pH 4.9 prior to the addition of the metal salts (Table 4.1). The long term monitoring results shows the pH of the soil has changed during the course of the experiment. The addition of metal chloride and nitrate salts (zinc chloride, copper chloride, cadmium chloride, nickel chloride and lead nitrate) to the soil has acidified the soils by between 0.1 and 0.4 pH units. The addition of potassium dichromate has reduced soil acidity by raising the soil pH by between 0.1 and 1.0 pH unit. The pH of the control soils have generally decreased for all treatments over the 26 months, however, little effect was observed after a period of 10 months.

The results indicate the pH changes are amplified with time by comparing the soil pH results from 10 months and 26 months. However, the control soils have also become more acidic with time. This increasing acidity may be due to the addition of irrigation water or the influence of tree growth on the soil.

4.2.4 Metal Concentrations in Soil Solution

4.2.4.1. Introduction

When a water soluble metal salt is added to a soil, the salt will dissolve in the soil solution. At time zero it is assumed all the added metal salt is soluble and available for plant uptake. Within a very short period of time some of this added metal will be removed from the soil solution. Metals will be held by cation exchange processes on clays and organic matter, complexed by organic matter, adsorbed on to oxides and precipitated. With the passing of time the fraction of metal available for plant uptake will decrease and the concentration of each metal in the soil solution will reach an equilibrium between the concentration in the soil solution and concentration held in the soil.

The use of selective extraction procedures to determine the concentration of metal in the soil solution has been deemed inappropriate in this instance because the excess volume of extractant added to the soil solution will change the volume of soil solution and upset the equilibrium. In this regard a procedure was devised to extract soil solution for analysis (Merian et al., 1980).

4.2.4.2. Procedure

Sub-samples of air dry and sieved soil (<2mm) were contaminated with calculated weights of metal salt and sufficient water added too give the soils a 50% moisture content. The concentration of metal in soil solution was the determined on extracts of soil solution.

The experiment was undertaken in accordance with the documented procedure detailed in Chapter 2, Section 2.3.8 and the soil solution extracts were analysed by Atomic Absorption in accordance with procedures documented in Section 2.5. The weight of each metal salt added to 20g of soil is given in Table 4.5.

Table 4.5. The weight of metal salt added to 20g of soil

Metal Salt	Soil Concentration ($mg\ g^{-1}$)	Wt. salt for 1g Metal (g)	Wt. salt for 20g soil
Zinc Chloride	500	2.08	0.0208g
	3000	2.08	0.1248g
Copper Chloride	500	2.11	0.0211g
	2000	2.11	0.0844g
Nickel Chloride	500	4.05	0.0405g
	1000	4.05	0.081g
Cadmium Chloride	300	2.03	0.0122g
	1000	2.03	0.0406g
Potassium Dichromate	2000	2.82	0.1128g
Lead Nitrate	2000	1.60	0.064g

4.2.4.3. Results

The results of the analysed metal concentrations in the soil solutions are tabulated in Table 4.6.

Table 4.6. Metal Concentrations in Air Dry Soil and Soil Solution at 50% Moisture

Soil Treatment	Soil Metal Concentration ($mg\ kg^{-1}$ Soil)	Soil Solution Concentration ($mg\ l^{-1}$)
500 Zinc	547	131
3000 Zinc	3026	1860
500 Copper	764	8
2000 Copper	2274	75
500 Nickel	548	249
1000 Nickel	1027	367
300 Cadmium	310	2
1000 Cadmium	1028	34
2000 Lead	2012	<20
2000 Chromium	2004	1140

4.2.4.4. Discussion

The test results of the soil metal treatments (500 Zn, 500 Cu, 2000 Cu, 300 Cd, 1000 Cd and 2000 Pb) indicate that of the total metal added less than 20% of the total metal added is present in the soil solution, for some of the above treatments the soil solution concentration is less than 5% of the total added. The test results for soil treatments

(3000 Zn, 500 Ni, 1000 Ni and 2000 Cr) indicate that between 33-50 % of the total added metal remains in the soil solution.

These results show that there are differences in the availability of individual metals added to the pot soil. These results are linked to the adsorption, chelation, chemisorption and precipitation behaviour of different metals in a similar soil. The results also show that increasing the concentration of a metal in the soil also increases the concentration of that metal in the soil solution. On the basis of the metal contents determined in the soil solution it would be logical to suggest that treatments 2000 Pb, 300 and 1000 Cd, 500 Zn and 500 and 2000 Cu should not be as toxic as the total concentrations would indicate.

4.2.5 Metal Adsorption

4.2.5.1. Introduction

A soil is comprised of many different components, including different sized particles derived from weathered rock, organic matter, inorganic components and biological components. It is therefore likely that no two soils are the same and therefore it is impossible to predict the behaviour of added metals in a given soil. It should be noted that different samples of the 'same' soil will also vary marginally in composition. The physical and chemical properties of the pot soil are given in Table 4.1 but the results are based on only a small sub-sample of soil.

In this regard the adsorption of metals in a soil can only be determined by laboratory analysis. Metal adsorption in a soil can occur through a number of soil processes including ion exchange, chemisorption, precipitation and chelation. The level of metal adsorption can be determined by shaking sub-samples of a given soil with different concentrations of a given metal solution. After a period a shaking the concentration of metal remaining in solution can be analysed and the concentration of metal adsorbed can be calculated. The results from such an experiment can be plotted

in graphical form and maximum adsorption values for individual metals in a given soil can be calculated.

4.2.5.2. Procedure

The experimental procedure including the preparation of individual metal solutions is discussed in Chapter 2, Section 2.3.7.

The calculated adsorption results for each metal (mg g^{-1} of air dry soil) were plotted against the equilibrium metal concentration (mg l^{-1}). The resulting graph is termed a adsorption isotherm. The adsorption isotherm has been used to derive maximum metal adsorption values.

For each metal a Langmuir isotherm was plotted comprising a plot of equilibrium concentration (mg l^{-1}) against equilibrium concentration divided by the weight of adsorbed metal.

The formula of the best fit line derived from the Langmuir isotherm was used to calculate maximum metal adsorption. The value can be compared with the value derived from the adsorption isotherm.

From the given formula of the line

$$y = mx + c \quad \textit{where m is the slope of the line}$$

and

$$\textit{Maximum Adsorption } (Xm = {}^{1}/_{Slope})$$

4.2.5.3. Results

The plotted results of the adsorption isotherms and Langmuir isotherms for the six metals (Cu, Cd, Zn, Ni, Cr and Pb) are shown in the following figures (Figure 4.1 to Figure 4.12). The maximum adsorption values for each metal are tabulated in Tables 4.7 and 4.8.

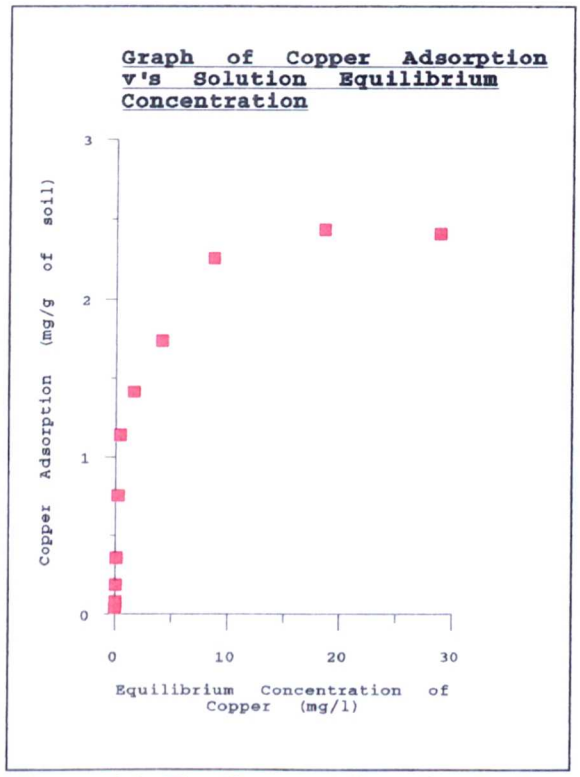


Figure 4.1. The Copper Adsorption Isotherm.

Figure 4.1. The Copper Adsorption Isotherm.

The copper solution was prepared from copper chloride [$CuCl_2.xH_2O$], the copper being present as the Cu^{2+} cation.

The maximum adsorption value for Cu, determined from the adsorption isotherm was 2.43 mg g^{-1} of soil.

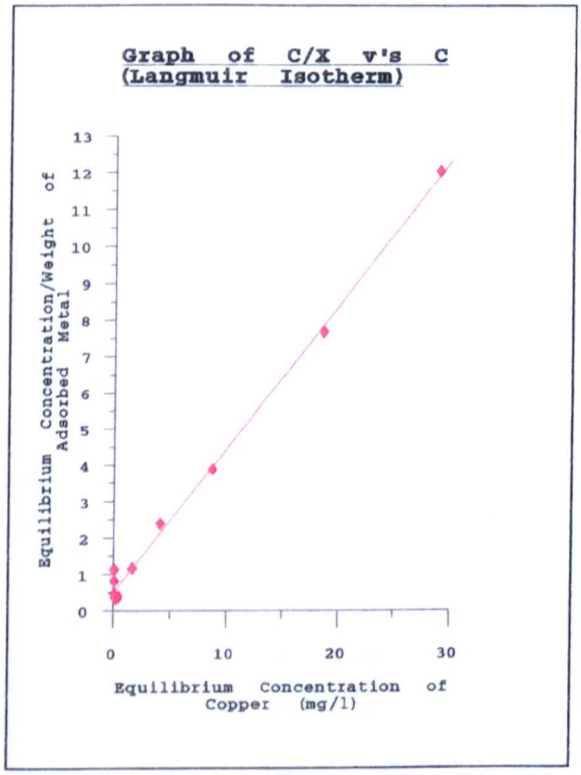


Figure 4.2. The Langmuir Isotherm for Copper Adsorption.

Figure 4.2 The Langmuir Isotherm for Copper Adsorption.

A best fit line was plotted through the respective points and the corresponding formula for the gradient of the line was:

$$y = 0.39x + 0.52$$

The maximum copper adsorption determined from the Langmuir isotherm was 2.56 mg g^{-1} of soil. This value generally agrees with the value of 2.43 mg g^{-1} determined from the copper adsorption isotherm

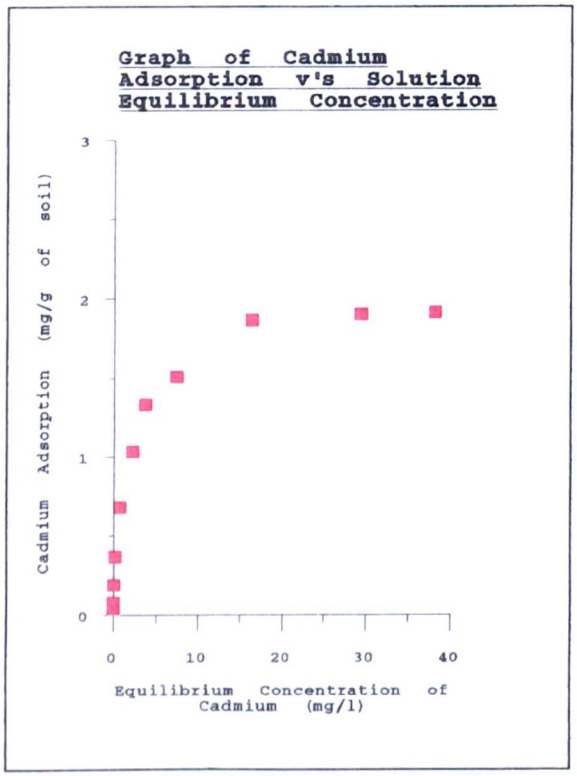


Figure 4.3. The Cadmium Adsorption Isotherm.

Figure 4.3 The Cadmium Adsorption Isotherm.

The cadmium solution was prepared from cadmium chloride salt [$CdCl_2.2^1/_2 H_2O$], the cadmium being present as the Cd^{2+} cation.

The maximum cadmium adsorption determined from the graph was 1.92 mg g^{-1} of soil.

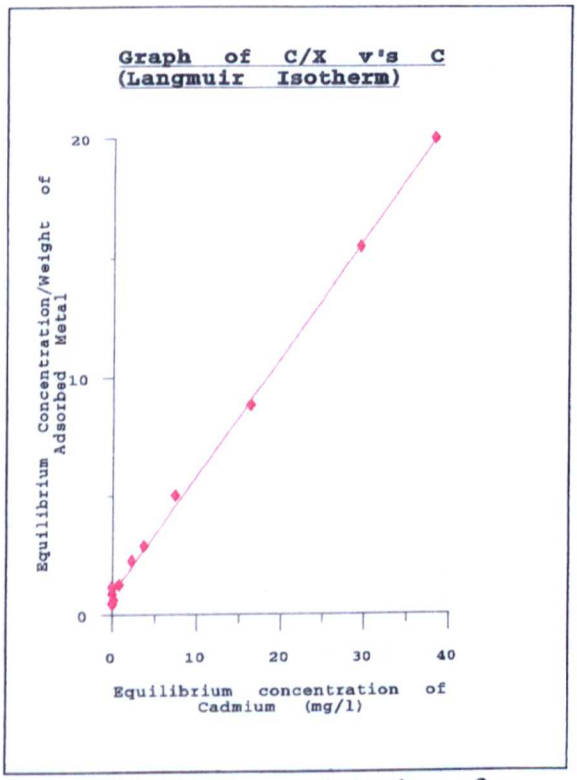


Figure 4.4. The Langmuir Isotherm for Cadmium Adsorption.

Figure 4.4 The Langmuir Isotherm for Cadmium Adsorption.

A best fit line was plotted through the respective points and the slope of the line given by the following formula

$$y = 0.50x + 0.83$$

The maximum cadmium adsorption value determined from the Langmuir isotherm was 2 mg g^{-1} of soil.

This value generally agrees with the value of 1.92 mg g^{-1} determined from the cadmium adsorption isotherm.

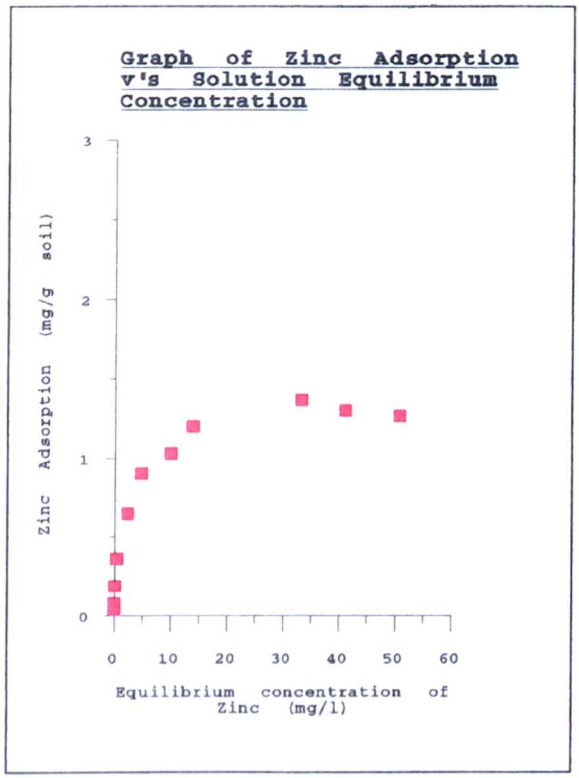


Figure 4.5. The Zinc Adsorption Isotherm.

Figure 4.5 The Zinc Adsorption Isotherm.

The zinc solution was prepared from zinc chloride salt [$ZnCl_2$], the zinc being present as the Zn^{2+} cation.

The maximum zinc adsorption value determined from the graph was 1.37 mg g^{-1} of soil.

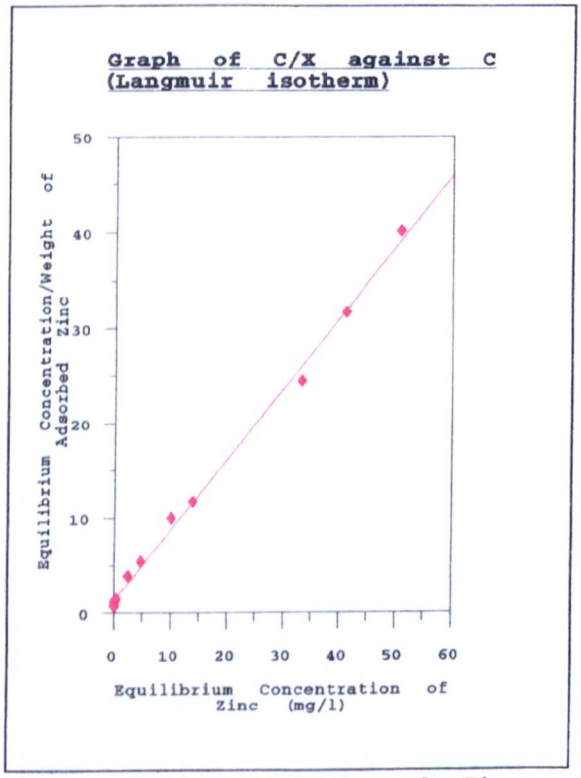


Figure 4.6. Langmuir Isotherm for Zinc Adsorption

Figure 4.6 The Langmuir Isotherm for Zinc Adsorption.

A best fit line was plotted through the respective points and the corresponding formula for the gradient of the line was as follows

$$y = 0.75x + 1.23$$

The maximum adsorption value determined from the Langmuir isotherm was 1.33 mg g^{-1} of soil.

This value generally agrees with the value of 1.37 mg g^{-1} determined from the zinc adsorption isotherm.

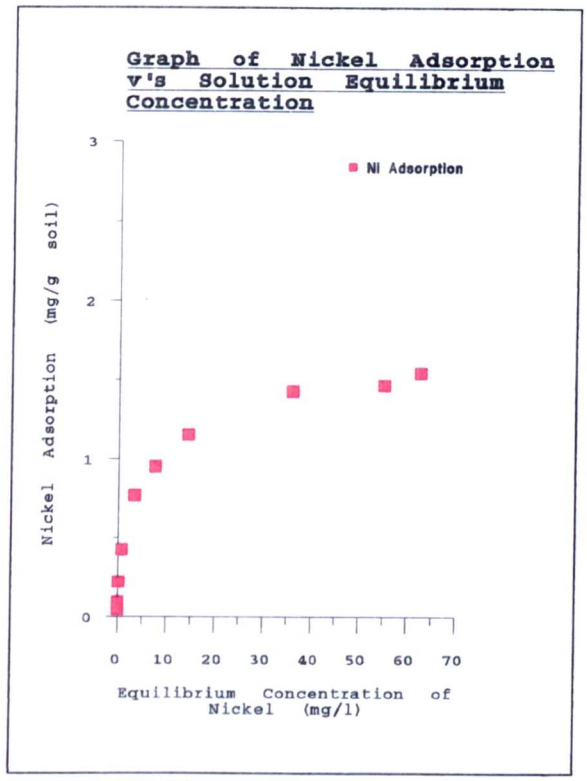


Figure 4.7. The Nickel Adsorption Isotherm.

Figure 4.7 The Nickel Adsorption Isotherm.

The nickel solution was prepared from nickel chloride [$NiCl_2.6H_2O$], the nickel being present as the Ni^{2+} cation.

The maximum nickel adsorption determined from the graph was 1.54 mg g^{-1} of soil.

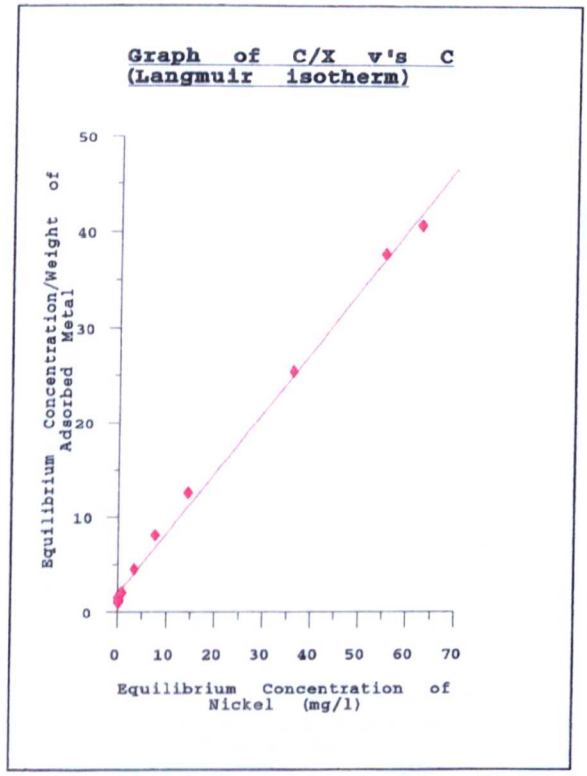


Figure 4.8. The Langmuir Isotherm for Nickel Adsorption

Figure 4.8 The Langmuir Isotherm for Nickel Adsorption.

A linear best fit line was plotted through the respective points and the corresponding formula for the line was

$$y = 0.64x + 1.89$$

The maximum nickel adsorption value determined from the Langmuir isotherm was 1.56 mg g^{-1} of soil.
This value agrees closely with the value of 1.54 mg g^{-1} determined from the nickel adsorption isotherm.

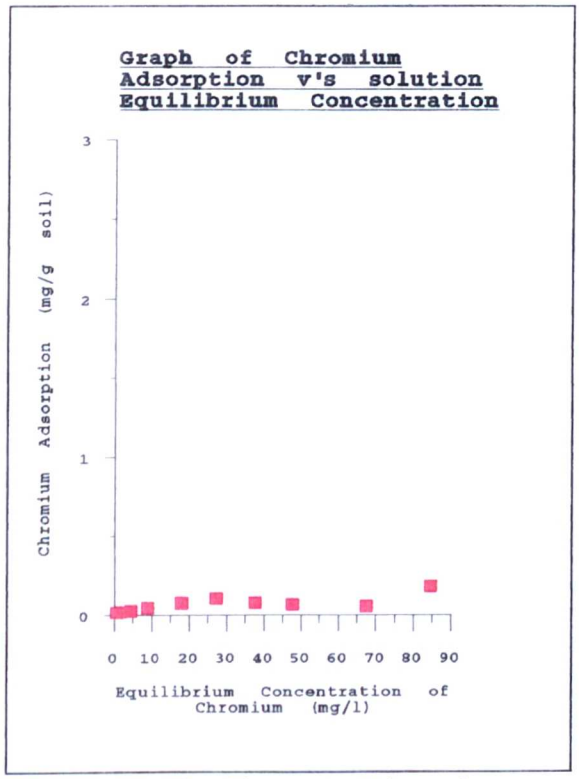


Figure 4.9. The Chromate Adsorption Isotherm.

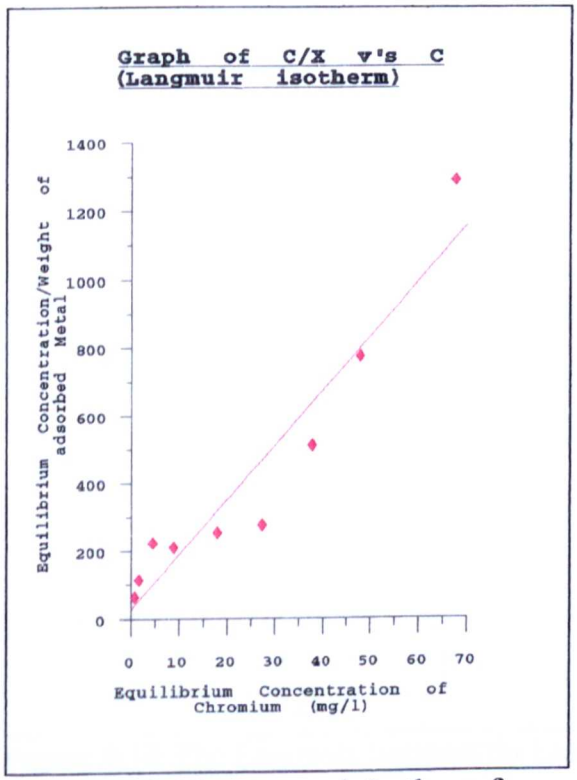


Figure 4.10. The Langmuir Isotherm for Chromate Adsorption.

Figure 4.9 The Chromate Adsorption Isotherm.

The chromium solutions were prepared from the potassium dichromate salt [$K_2Cr_2O_7$], the chromium being present as the chromate anion.

The maximum adsorption value for chromate determined from the graph was 0.18 mg g^{-1} of soil.

Figure 4.10 The Langmuir Isotherm for Chromate Adsorption.

A linear best fit line was plotted through the respective points and the corresponding formula for the gradient of the line was given as;

$$y = 16.15x + 27.11$$

The maximum calculated chromium adsorption value determined from the Langmuir isotherm was 0.062 mg g^{-1} and a value of 0.18 mg g^{-1} was determined from the chromate adsorption isotherm. The discrepancy in the results is probably due to the very low concentrations of chromate adsorbed.

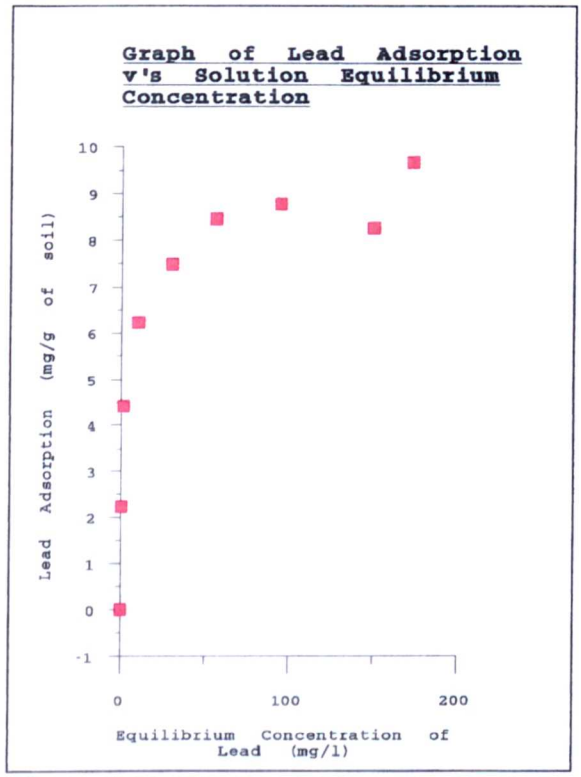


Figure 4.11. The Lead Adsorption Isotherm.

Figure 4.11 The Lead Adsorption Isotherm.

The lead solution was prepared from lead nitrate [$Pb(NO_3)$], the lead being present as the Pb^{2+} cation.

A maximum lead adsorption value of 9.64 mg g^{-1} of soil was determined from the graph.

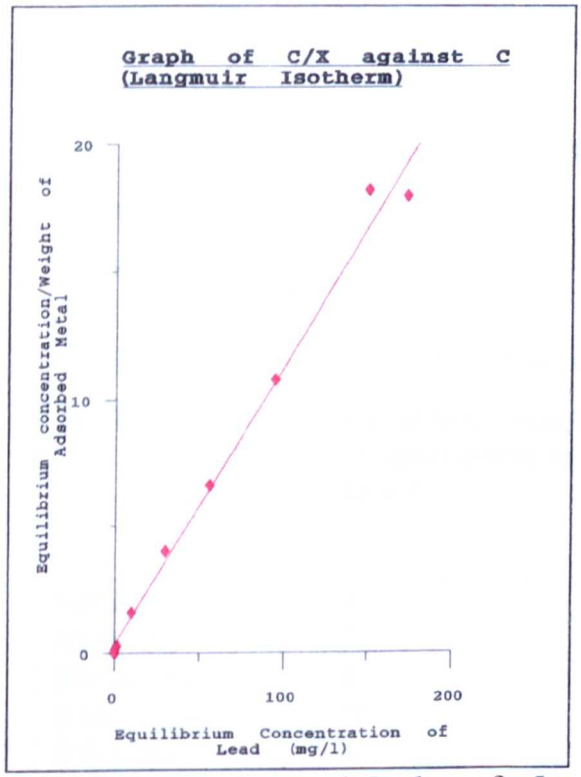


Figure 4.12. The Langmuir Isotherm for Lead Adsorption

Figure 4.12 The Langmuir Isotherm for Lead Adsorption.

A linear best fit line was plotted through the respective points and the corresponding formula for the best fit line was;

$$y = 0.11x + 0.34$$

The maximum adsorption value for lead determined from the Langmuir isotherm was 9.09 mg g^{-1} of soil. This value generally agrees with the value of 9.64 mg g^{-1} of soil determined from the lead adsorption isotherm.

The calculated maximum adsorption values for each metal determined from the adsorption and Langmuir isotherms are shown in Table 4.7.

Table 4.7. Maximum Metal Adsorption

Heavy Metal	Maximum Adsorption (adsorption isotherm)	Maximum Adsorption (Langmuir Isotherm)
zinc	1.37	1.33
copper	2.43	2.56
nickel	1.54	1.56
cadmium	1.92	2.00
lead	9.64	9.09
chromium	0.18	0.06

NOTE: All values given are expressed as mg g^{-1} of soil

Given the maximum metal adsorption levels determined (Table 4.7, Langmuir Isotherm) and the weight of soil identified in each pot treatment the theoretical quantity of metal adsorbed by 8 kg of 'pot' soil has been calculated. As discussed by Wild (1988) the main components of soil involved with adsorption reactions are present in the less than 2 mm sieved fraction. In this regard a further calculation has also been undertaken to determine the maximum adsorption of metal on the less than 2 mm soil fraction. The value obtained from the particle size fractionation in Table 4.1 (47.4%) has been used. These calculated maximum adsorptions are shown in Table 4.8.

Table 4.8. The Maximum Adsorption of Metal (Pot Soil)

Soil Treatment	Calculated weight of metal added to 8 kg pot	Theoretical maximum adsorption in 8 kg of soil	Maximum adsorption in < 2 mm soil fraction
500 Zn	4	10.6	5.0
3000 zinc	24	10.6	5.0
500 copper	4	20.5	9.7
2000 copper	16	20.5	9.7
500 nickel	4	12.5	5.9
1000 nickel	8	12.5	5.9
300 cadmium	2.4	16.0	7.6
1000 cadmium	8.0	16.0	7.6
2000 chromium	16	0.50	0.23
2000 lead	16	72.7	34.5

NOTE: All values are expressed as grams (g)

4.2.5.4. Discussion

The adsorption isotherms for all the metals concerned (Cd, Cr, Cu, Pb, Ni and Zn) confirm that there are differences in soil adsorption behaviour between the different metals. The chemical nature of the added metal also affects the adsorption of the added metal as shown with chromium. The chromate anion carries a negative charge and as measured little chromium was adsorbed by the soil.

All the metal elements added as cations showed variable degrees of adsorption. The order of adsorption was Pb >> Cu > Cd > Ni > Zn and the calculated maximum adsorption values were 9.09, 2.56, 2.00, 1.56 and 1.33 mg of metal g^{-1} of air dry soil respectively (Langmuir isotherm).

Chromium was added as the anionic dichromate ($Cr_2O_7^{2-}$). Due to the negative charge the ion did not show the typical surface adsorption characteristics onto exchange sites and hydrous oxides. The maximum calculated adsorption value for chromate was 0.062 mg g^{-1} of soil. As the chromate anions should be attracted to sites of positive charge the findings suggest there are very few sites of positive charge in the soil.

It is important to note that the weight of each metal salt added to each pot was calculated on the basis of 8 kg of pot soil. However, under laboratory conditions metal adsorption reactions are undertaken on soil passing through a 2 mm sieve. In this regard the < 2 mm faction of soil in each pot is around 50% of the total and as a result the weight of metal adsorbed by the pot soil may be less than anticipated.

These results suggest that when artificial spoils are prepared to give comparable total soil metal concentrations, metal availability and concentrations in the soil solution will vary. From the results the availability of the six added metals to the tree seedlings will be in the order of Cr > Zn >Ni > Cd > Cu > Pb.

It should be noted that these adsorption values were calculated over a period of 24 hours for all metals concerned. In the pot experiment the time period will be longer (up to $2^1/_2$ years) and the quantity of metal adsorbed by the soil should increase

with time. Soil processes which adsorb and remove added metal include specific adsorption and chemisorption, precipitation, immobilisation by soil organisms, further exchange on cation exchange sites, chelation and specific sorption. A quantity of metal may also be removed by the tree seedlings and some soil solution metal may be leached from the soil by irrigation water.

4.3 TREE SEEDLING SURVIVAL

4.3.1 Introduction

In an ideal situation the preferred option would have been to contaminate soils with heavy metal salts up to 6 months prior to planting the tree seedlings. This procedure would have allowed the metal and soil to reach an equilibrium prior to planting. Given the relatively short period of time to implement the pot experiment there was insufficient time to adopt the preferred option. As detailed in Chapter 2, Section 2.6 the tree seedlings were planted directly in to the prepared soil/metal mix.

Due to this procedure the fatality rate of the planted tree seedlings was expected to be higher than if the seedlings had been planted in a soil/metal mix which had reached equilibrium. The survival of all seedlings was assessed at the end of the first and second growing seasons. All dead seedlings were replaced in the winter period between the first and second growing season with identical seedlings obtained from the original supplier.

4.3.2 Procedure

A visual assessment was made on the survival of all transplanted tree seedling at various times during the duration of the pot experiment. The assessment was based on observations made with regard to various green colouration on the foliage. Survival assessments were made on the 7 July 1994, 11 February 1995, 20

November 1995 and 19 August 1996. The seedlings assessed as dead on the 11 February 1995 were replaced with new seedlings.

4.3.3 Results

The health of each seedling was assessed visually in the autumn of 1995 and 1996. Generally a seedling which had failed to produce leaves or new shoots was assessed as dead.

The survival rates given assume all the seedlings were healthy when transplanted into the respective contaminated soils. The survival rates are expressed as percentages of number of seedlings living per treatment against total number of trees planted for that treatment. The results of the survival assessment are tabulated in Tables 4.9 (a to d).

Table 4.9a The Percentage Survival Rates of *Salix viminalis*

Soil Metal Treatments	Assessment Dates					
	First Planting			Replanting		
	Mar-94	Jul-94	Feb-95	Mar-95	Nov-95	Aug-96
500 zinc	100	80	20	100	90	0
3000 zinc	100	0	0	100	0	0
500 copper	100	90	90	100	90	90
2000 copper	100	0	0	100	30	0
500 nickel	100	10	0	100	30	0
1000 nickel	100	10	0	100	10	0
300 cadmium	100	100	100	100	70	30
1000 cadmium	100	40	20	100	0	0
2000 lead	100	50	50	100	30	30
2000 chromium	100	0	0	100	10	0
control	100	96	92	100	92	92

Table 4.9b The Percentage Survival Rates of *Pinus contorta*

Soil Metal Treatments	Assessment Dates					
	First Planting			Replanting		
	Mar-94	Jul-94	Feb-95	Mar-95	Nov-95	Aug-96
500 zinc	100	90	40	100	80	30
3000 zinc	100	40	0	100	0	0
500 copper	100	90	70	100	90	60
2000 copper	100	20	0	100	0	0
500 nickel	100	80	0	100	0	0
1000 nickel	100	40	0	100	0	0
300 cadmium	100	100	60	100	40	20
1000 cadmium	100	50	0	100	10	0
2000 lead	100	40	20	100	20	0
2000 chromium	100	30	0	100	0	0
control	100	100	92	100	100	100

Table 4.9c The Percentage Survival Rates of *Alnus incana*

Soil Metal Treatments	Assessment Dates					
	First Planting			Replanting		
	Mar-94	Jul-94	Feb-95	Mar-95	Nov-95	Aug-96
500 zinc	100	40	0	100	30	20
3000 zinc	100	0	0	100	0	0
500 copper	100	30	0	100	70	80
2000 copper	100	0	0	100	0	0
500 nickel	100	10	0	100	20	0
1000 nickel	100	10	10	100	0	0
300 cadmium	100	50	0	100	50	70
1000 cadmium	100	10	0	100	0	0
2000 lead	100	40	30	100	50	40
2000 chromium	100	10	0	100	0	10
control	100	92	92	100	100	88

Table 4.9d The Percentage Survival Rates of *Populus trichocarpa*

Soil Metal Treatments	Assessment Dates					
	First Planting			Replanting		
	Mar-94	Jul-94	Feb-95	Mar-95	Nov-95	Aug-96
500 zinc	100	100	100	100	20	0
3000 zinc	100	0	0	100	0	0
500 copper	100	90	70	100	40	20
2000 copper	100	0	0	100	20	0
500 nickel	100	40	0	100	10	10
1000 nickel	100	0	0	100	0	0
300 cadmium	100	100	60	100	0	0
1000 cadmium	100	30	0	100	0	0
2000 lead	100	50	50	100	20	20
2000 chromium	100	0	0	100	0	0
control	100	100	100	100	88	82

4.3.4 Discussion

The tree seedlings subjected to the metal treatments showed higher fatality rates when compared with survival rates in the controls. Soil treatments (3000 Zn, 2000 Cu, 500 and 1000 Ni and 2000 Cr) were toxic to all the study trees. Initial assessments made on the seedlings subjected to these treatments recorded a few surviving trees but these appear to have died soon afterwards.

Better survival rates were recorded in soil treatments 500 Zn, 500 Cu, 300 Cd and 2000 Pb. These metal treatments had relatively low soil solution metal concentrations (131, 8, 2 and 0 mg l^{-1}) respectively and all had salinity values less than 2000 $\mu S\ cm^{-1}$.

In conclusion the survival rates of the study seedlings appear to be linked closely with the salinity of the soil solution and the soil solution metal concentration.

4.4 SELECTIVE EXTRACTION OF POT SOIL

4.4.1 Introduction

Previous metal adsorption studies (Section 4.2.5) showed that when a metal salt was added to the study soil some of the metal was adsorbed by the soil and removed from soil solution. With time the availability of the added metal decreases.

In an attempt to quantify the change in availability of added metal, the 'pot' soils were sampled at intervals of 10 and 26 months after the addition of each metal salt (the soil samples were collected during February 1995 and August 1996). The samples were analysed by non-sequential selective extraction procedures using 0.01M calcium chloride and 0.05M ammonium EDTA. The 0.01M calcium chloride was selected to remove the fraction of soil metal residing in the soil solution and held on cation exchange sites, 0.05M ammonium EDTA removed the organically bound fraction from the soil. The residual metal content of the soils was determined by acid digestion.

4.4.2 Procedure

The soil samples were collected in accordance with the documented procedures detailed in Chapter 2, Section 2.1.3.

The selective extraction was carried out in accordance with documented procedures using 0.01M calcium chloride and 0.05M ammonium EDTA, refer Chapter 2, Section 2.3.10. Residual metal was determined by acid digest of soil as documented in Section 2.3.9.

4.4.3 Results

The results of the selective extraction of the pot soils with 0.01M calcium chloride and 0.05M ammonium EDTA are summarised in Tables 4.10 and 4.11 respectively. The acid digest results are summarised in Table 4.12. The calculated standard deviation values are included as ± values. These tables are included in Appendix 2.

The results are expressed as soil metal concentrations extracted from air dry and sieved soil (< 2 mm). In this regard it is worth noting that the soil in each experimental pot also contained 52.6% material with a particle size > 2 mm (Table 4.1). Such material is often considered to have little influence on a soils chemical properties. In this regard it could be assumed that all the metal added to each pot is held within the <2 mm fraction. The soil metal concentrations for each soil treatment have been averaged and are shown in Figure 4.13 'Selective Extraction of Pot Soil'. Figure 4.14 'Selective Extraction of Pot Soil (Amended)' shows the same results amended to reflect average metal concentrations in the combined < 2 and > 2 mm soil fractions (ie. soil prior to sieving).

Figure 4.13 Selective Extraction of Pot Soil

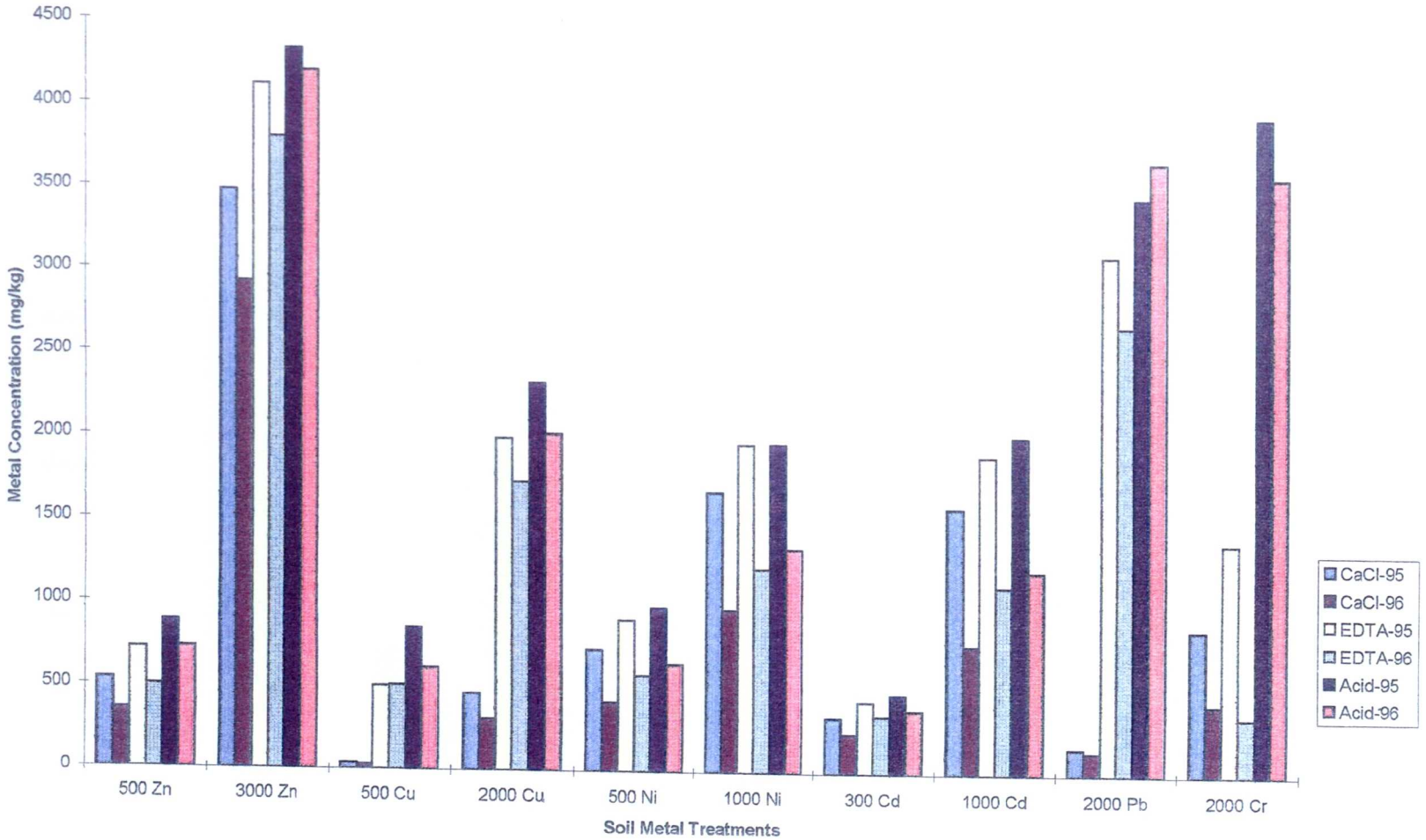
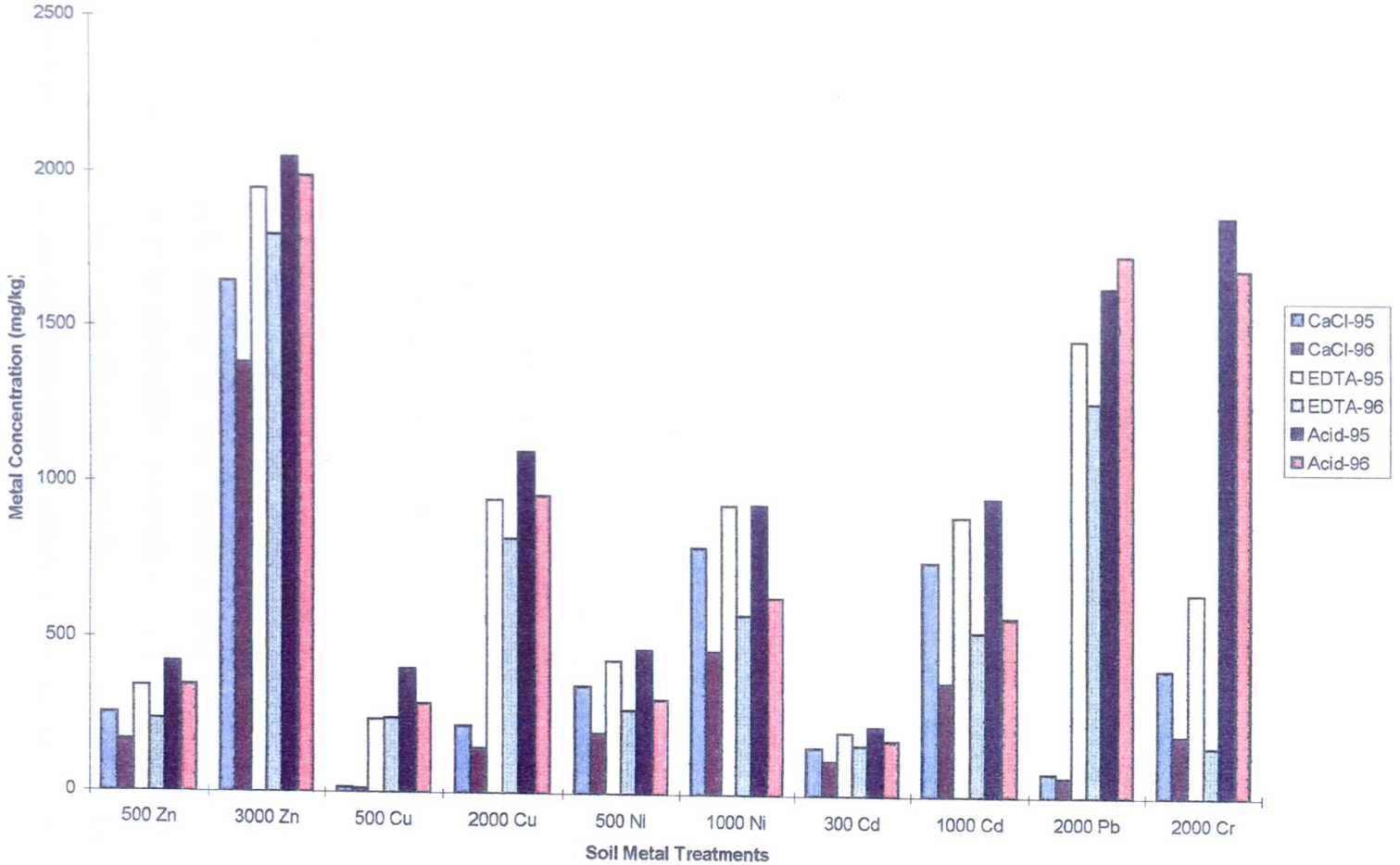


Figure 4.14 Selective Extraction of Pot Soil (Amended)



4.4.4 Discussion

The tabulated values in Tables 4.10, 4.11 and 4.12 show the average concentration of metal removed from each artificially contaminated soil for each species of tree on two separate sampling dates.

For comparison the concentrations of metal extracted by each procedure have been presented in Figure 4.13. The values have been adjusted to take account of metal present in the control soil.

The order of metal removal from the soil from the highest concentrations of metal extracted to the lowest was acid digest > ammonium EDTA > calcium chloride. That is to say that ammonium EDTA extracted higher concentrations of metal than calcium chloride. The results therefore confirm the expected results on the basis of the order and selection of extracting agents.

The residual metal concentrations measured in the < 2mm soil fraction were in the order of 60 to 70% higher than the intended soil metal concentrations. However, when the results were amended to take into consideration the fraction of soil > 2mm the soil concentrations are slightly less than the intended soil concentrations (Figure 4.14). The results indicate that most of the metal is held within the < 2mm soil fraction. As discussed, the > 2mm soil fraction has little influence on a soils chemical properties and that is why it is removed by sieving during soil preparation (MAFF, 1986).

In almost all treatments (excluding lead) the total residual metal concentration in the soil was higher during the first sampling when compared with the second sampling. This may be attributable to sampling and analytical discrepancies but it is likely some metal will have been leached from the soil or taken up by the seedlings. The results are likely to be a combination of the above factors.

The calcium chloride solution extracted more metal from the soils collected after 10 months when compared with the 26 month samples. These findings may

indicate the adsorption, chemisorption and precipitation of metal within the soil and as a factor of time more of the added metal becomes more strongly held in the soil.

The ammonium EDTA extractions removed higher percentages of copper and lead when compared with the percentage of the other metals removed by calcium chloride. This may indicate that lead and copper are strongly bound on soil organic matter or are more strongly bound on some other soil component.

The calcium chloride extraction indicates that the order of availability as a percentage of the total soil metal, starting with the most available was 1000 Ni > 3000 Zn > 500 Ni > 300 Cd > 500 Zn > 2000 Cu > 500 Cu >2000 Pb. In this regard 1000 Ni and 3000 Zn should be more toxic than the 500 Cu and 2000 Pb treatments.

4.5 FOLIAGE ANALYSIS

4.5.1 Introduction

The pot experiment was not designed to be a sacrificial experiment whereby complete trees were harvested at selected periods through the growing season. Initially an allowance had been made for the collection of foliage samples every month throughout the growing season from each tree species and soil metal treatment.

However, due to the poor survival rates of the seedlings it was not possible to undertake such an intensive sampling programme and therefore biomass samples were collected at intermittent periods throughout the duration of the experiment.

4.5.2 Procedure

Foliage samples were hand picked from a number of identical soil metal treatments and plant species. The samples were collected into polythene bags and were clearly labeled to identify species, the date of collection and soil metal treatment. The samples were prepared and analysed in accordance with documented procedures given in Chapter 2, Sections 2.4 and 2.5.

Due to the poor survival rates of some seedlings it was decided to scale down the frequency of sampling from that initially proposed. Foliage samples were collected during June 1994, June 1995 and August 1996. It should be noted no foliage samples could be collected from seedlings which did not produce new growth, in some instances badly wilted leaves were collected whenever possible.

At the end of the research project the surviving seedlings in each treatment were harvested and divided into individual components of wood, bark and leaves which were analysed for total metal content of each treatment metal by acid digest.

4.5.3 Results

The metal contents of foliage collected from the four study species are tabulated in Table 4.13 included in Appendix 3. The maximum concentrations of each metal recorded are presented in Figure 4.15 'Maximum Metal Concentrations in Foliage'. The results presented include the subtraction of the repective metal concentration recorded in the control species.

The results are tabulated in Table 4.14 included in Appendix 3. The recorded metal concentrations in different components of *Alnus incana* are shown in Figure 4.16 'Metal Concentration in Different Components of *Alnus incana*'.

Figure 4.15 Maximum Metal Concentrations in Foliage

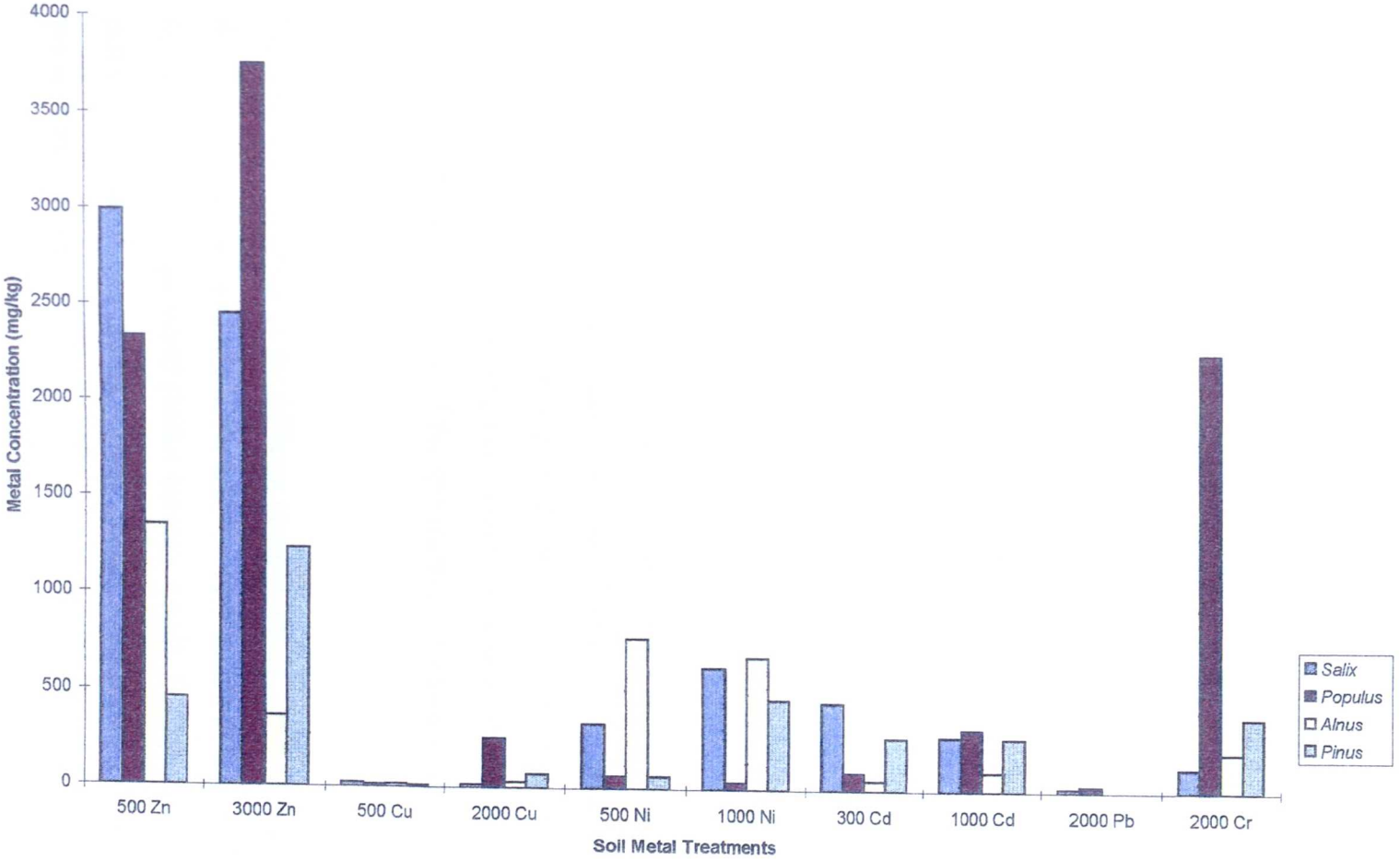
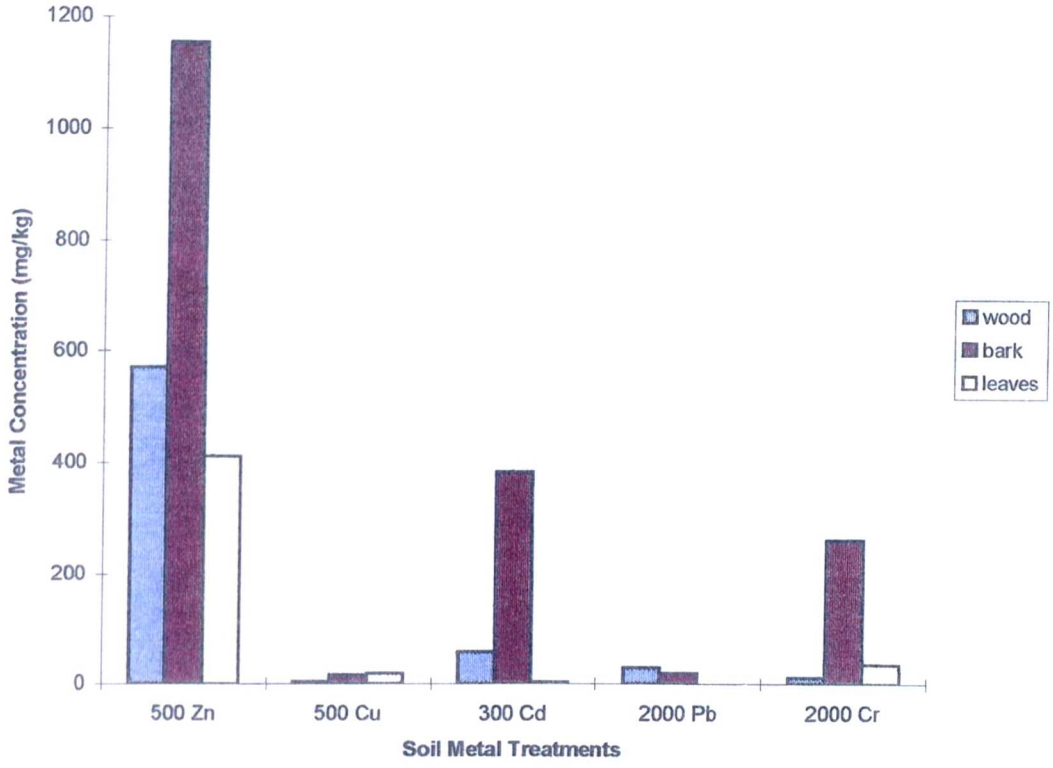


Figure 4.16 Metal Concentration in Different Components of *Alnus incana*



4.5.4 Discussion

The analysis of the biomass samples collected from the 'Pot' experiment (Figure 4.15) show that the maximum levels of zinc (3756 mg kg^{-1}) , nickel (779 mg kg^{-1}), cadmium (453 mg kg^{-1}) and chromium (2274 mg kg^{-1}) have been transported to the foliage of the study species, while lower levels of lead and copper were analysed in foliage. Metal uptake in most of the species was increased by increasing soil metal concentrations.

From the metal analysis of the different components of *Alnus incana* (Figure 4.16), the metals; chromium and to a lesser extent copper accumulate in the bark followed by the leaves then the wood (bark> leaves> wood). For zinc and chromium the pattern of accumulation is bark> wood> leaves. Lead shows a different pattern of wood> bark> leaves.

It is also important to note differences in uptake between different species, poplar accumulated more chromium and zinc than any other species, alder more nickel and willow more cadmium. These results can be confusing as in the first year willow accumulated more zinc but in the second year it was poplar which showed higher accumulation. These complicated results may be due to the unsettled nature of the soil.

The use of trees for the remediation of heavy metal contaminated soils requires the selection of trees which are both tolerant and capable of accumulating significant levels of metal. Metal accumulation rates can be expressed as a ratio of the total metal content of biomass versus the total elemental content in the soil in which the plant was growing. It has been suggested where the ratio value exceeds a value of 1, the subject plant is displaying hyper-accumulation of the metal in question (Raskin et al., 1994).

In this regard the metal concentrations determined in seedling foliage (Table 4.13) and the residual metal content of the respective soils (Table 4.12) have been used to assess the seedling/soil uptake ratio. The uptake ratio is determined by dividing the maximum concentration in the foliage by the residual metal content in the soil.(Table 4.15).

Table 4.15. Plant Metal Uptake Ratio

Soil Treatment	Plant/ Soil Uptake Ratio			
	Poplar	Pine	Willow	Alder
500 Zn	3.1	0.60	3.4	1.8
3000 Zn	0.9	0.3	0.6	0.1
500 Cu	0.01	0.01	0.02	0.02
2000 Cu	0.1	0.03	0.03	0.01
500 Ni	0.06	0.06	0.4	0.9
1000 Ni	0.02	0.2	0.4	0.4
300 Cd	0.2	0.4	1.0	0.1
1000 Cd	0.2	0.1	0.1	0.05
2000 Pb	0.003	0.002	0.002	0.002
2000 Cr	0.6	0.05	0.04	0.04

In almost all instances increasing the soil metal concentration results in an increased concentration of the metal within the plant tissue, however, the calculated uptake ratios tend to be less.

The order of uptake starting with the metal which displayed the highest level of uptake was Zn>> Cd, Cr and Ni > Cu >Pb. The 500 mg kg^{-1} zinc soil treatments with *Populus trichocarpa, Salix viminalis* and *Alnus incana* gave uptake ratios of 3.1, 3.4 and 1.8 respectively. A uptake ratio of 1.0 was recorded for the soil treatment of 300 mg kg^{-1} cadmium and *Salix viminalis.*

4.6 CONCLUSIONS

In summary the 'Pot' experiment did not provide as much information on the uptake of heavy metals as had been initially hoped. This was partly due to the poor survival of the tree seedling to the metal treatments used. From an initial assessment of the results it could be concluded that the selected metal concentrations for the soils were too toxic for the study species. The soil metal concentrations were selected following a review of the literature and the results of the soil metal concentrations determined in the field and were similar to metal levels determined in forest soils contaminated by smelter emissions.

Some authors had reported trees growing in soils with maximum metal concentrations of 10,000 mg kg^{-1} zinc, 9700 mg kg^{-1} copper, 50 mg kg^{-1} cadmium, 12,000 mg kg^{-1} lead and 5000 mg kg^{-1} nickel (Little and Martin, 1972, Hawrys, 1984, Hazlett et al., 1983 , Hogan and Wotton, 1984 and Tyler 1984). Apart from cadmium the metal concentrations used in the 'pot' experiment were lower than those reported. Smilde (1981) did report vegetation growth reductions at soil/suldge treatments of 300 mg kg^{-1} cadmium, 250-700 mg kg^{-1} nickel and 1000-3000 mg kg^{-1} copper and zinc. It is also worth noting that total metal concentrations determined in the 'field' soils were in excess of the levels in the pots. In this regard the total metal concentrations selected would not appear excessive in the light of other studies.

The results of the analysed metal concentrations in soil solution obtained by different procedures are presented in Table 4.16. The first column titled 'Soil Solution Metal ($CaCl_2$)' refers to the metal concentrations determined by the calcium chloride extraction of the prepared and sieved soil. The second column 'Soil Solution Metal ($CaCl_2$ Amended)' presents the results amended to reflect metal concentrations in the combined < 2mm and > 2mm soil fractions prior to sieving. The third column presents the results of the solution metal concentrations determined on an extracted volume of soil solution at 50% moisture.

Table 4.16. Soil Solution Concentrations

Soil Treatment	Soil Solution Metal ($CaCl_2$)	Soil Solution Metal ($CaCl_2$ Amended)	Soil Solution Metal
500 Zinc	566	269	131
3000 Zinc	3192	1519	1860
500 Copper	25	12	8
2000 Copper	373	177	75
500 Nickel	736	350	249
1000 Nickel	1430	680	367
300 Cadmium	339	161	2
1000 Cadmium	1655	788	34
2000 Lead	138	66	<20
2000 Chromium	513	244	1140

From the above results the metal concentrations assessed in the soil solution for metal treatments 2000 Pb, 300 Cd and 500 Cu were low when assessed by both methods. Survival rates indicate that it was these treatments which contained the highest survival rates. Unfortunately the 2000 Pb, 300 Cd and 500 Cu values for salinity in 1996 were also low, so that survival rates were (roughly) equally correlated with both low metal concentrations and low salinity.

During the initial selection of metal salts and concentrations, the influence of soil salinity was overlooked. From the studies undertaken on soil salinity there appears to a link between soil salinity, metal concentrations in the soil solution and tree seedling survival. In hindsight further studies should have been taken to determine the maximum levels of metal salt which would have been acceptable in accordance with guidance issued by MAFF (1988).

Conditions of high soil salinity are less likely to arise in contaminated 'field' soils because excess soluble metal will be leached from the soil which will reduce soil salinity. Furthermore, plant establishment would be unlikely before at least a thin upper 'leached' layer of soil had developed. The process was prevented in the 'pot' experiment by the watering regime adopted.

The foliage analysis results confirmed possible hyperaccumulation of zinc by poplar, willow and alder, while relatively high uptake levels of nickel, cadmium and chromium indicate the potential for using trees to phytoremediate soil contaminated with these metals. The results indicate that higher levels of metal uptake were recorded in the 'pot' experiment compared with the values obtained in the field.

The importance of the above studies will be discussed further in Chapter 6.

CHAPTER 5
HYDROPONICS

5.1 INTRODUCTION

Early studies of the mineral nutrition of plants by plant physiologists found that plants required a number of chemical elements for growth. During the 18th and 19th centuries researchers found that the nutrient requirement of green plants could be provided by a number simple inorganic salts or compounds These inorganic salts could be dissolved in water and the plants grown with their roots immersed in the solutions (Hershey, 1984).

The benefit of studying plant growth in nutrient solutions was first recognised when studying deficiency symptoms caused by the lack of essential nutrients. Selected nutrients could be omitted from nutrient solutions to induce deficiency symptoms in study plants. These initial studies were important in identifying two classes of nutrients, those nutrients which are required in relatively large quantities and are termed macro or major nutrients and those required in minute quantities termed micro or trace nutrients (Wild, 1988).

Nutrient solutions can be modified to study the effects of heavy metals on the growth of plants. In addition to dissolving macro and micro nutrients in solution, inorganic heavy metal salts can be dissolved in solutions. The main benefits of studying the effects of heavy metals on plants grown in nutrient solutions are as follows (Beauford et al., 1977):

- Heavy metal concentrations in the nutrient solutions can be closely controlled by the addition of the correct quantity and type of inorganic salt
- The effect of a single metal, or the additive effect of two or more metals can be studied.
- Experimental environmental factors can be controlled throughout the duration of the experiment (light, temperature, humidity and day length).

The definition of nutrient studies is wide ranging and can consist of system where the study plant is suspended with the roots immersed in a solution containing the necessary plant nutrients and the study metals are dissolved in the nutrient solution

(Sharma & Sharma, 1993, Dushenkov et al., 1995, Kumar et al., 1995, Jones et al., 1973, Beauford et al., 1977 and Moral et al., 1994). In other nutrient solution studies study plants have been grown in washed quartz sand and the metals and nutrients have been added in irrigation nutrient solutions during the watering regime (Kahle, 1993, Smith & Brennan, 1984 and Breckle & Kahle, 1992) or the germination of seed has been studied on filter papers impregnated with nutrient and metal solutions (Patterson & Olson, 1983).

Studies which have set out to study growth and metal toxicity symptoms have generally used relatively low concentrations of metal in nutrient solutions. Sharma & Sharma (1993) when studying the effects of chromium on wheat (*Triticum aestivum*) in nutrient solutions added chromium as sodium dichromate at levels of 0.05, 0.1, 0.25 and 0.5 mM. Scherbatskoy et al. (1986) studied the effects of acidity (pH 3,4 and 5) in conjunction with cadmium (1 mg l^{-1}), copper (5 & 10 mg l^{-1}), lead (5 & 20 mg l^{-1}) and zinc (5 & 10 mg l^{-1}).

As such hydroponic studies have developed particularly in the study of hyperaccumulation of heavy metals, higher solution concentrations have been used. A study by Kumar et al. (1995) highlighted the phytoremediation potential of *Brassica juncea* (L.) Czern which accumulates Pb, Cr, Cd, Ni, Zn and Cu in roots and shoots. Various metal solution concentrations were used: Cr (50 mg l^{-1}), Ni (100 mg $^{-1}$), Zn (100 mg l^{-1}) and Pb (500 mg l^{-1}). The metals were added as nitrate salts and potassium dichromate. The recorded maximum uptake of lead was 10,300 and 103,500 mg kg^{-1} dry weight in shoots and roots respectively.

With reference to these previous studies three metal concentrations of 10, 100 and 500 mg l^{-1} were chosen for each metal treatment to cover a wide range of metal concentrations. In addition the nutrient solution experiment was based on the suspension of seedlings with the roots immersed in nutrient solution to study the effects of six different heavy metals on nine different species of tree seedling.

5.2 ANALYSIS OF PLANT BIOMASS

5.2.1 Introduction

Details of the experimental design including the preparation of nutrient solution, the selection of tree seedlings, experimental setup and sampling are detailed in Chapter 2, Section 2.7.

5.2.2 Procedure

Foliage samples were collected at day 1, day 4, and day 7 and were harvested at between 15 and 31 days after the addition of the metal salts (Table 2.10). The final sampling date varied because of the volume of biomass samples to be segregated, sorted and dried at the end of the experiment. It was not possible to harvest all vegetation on one date and therefore the final sampling date varied for metal and concentration treatments. It should be noted that all plants within the same treatment were harvested at the same time.

5.2.3 Results

All biomass samples collected during the sampling programme were analysed for total metal content in accordance with documented procedures (refer Section 2.4). The results are presented in Tables 5.1 to 5.6 included in Appendix 4. For some treatments and dates no samples were collected due to lack of sample biomass and in this regard no results have been provided. The results are the mean value of duplicate analysis and the Standard Deviation of the results have been included as ± values. The limit of quantification is given in Section 2.5.4. Further reference should also be made to Section 2.5.5 'Certified Reference Material' which discusses the reproducibility of the analytical result as ± 7, 3, 5, 5, 10 and 2 mg kg^{-1} for zinc, copper, chromium, lead, nickel and cadmium respectively.

The results from the final harvesting date have been further presented in the following Figures 5.1 to 5.12. The values represent mean values of the analytical replicates.

Figure 5.1 Chromium Concentrations in Foliage

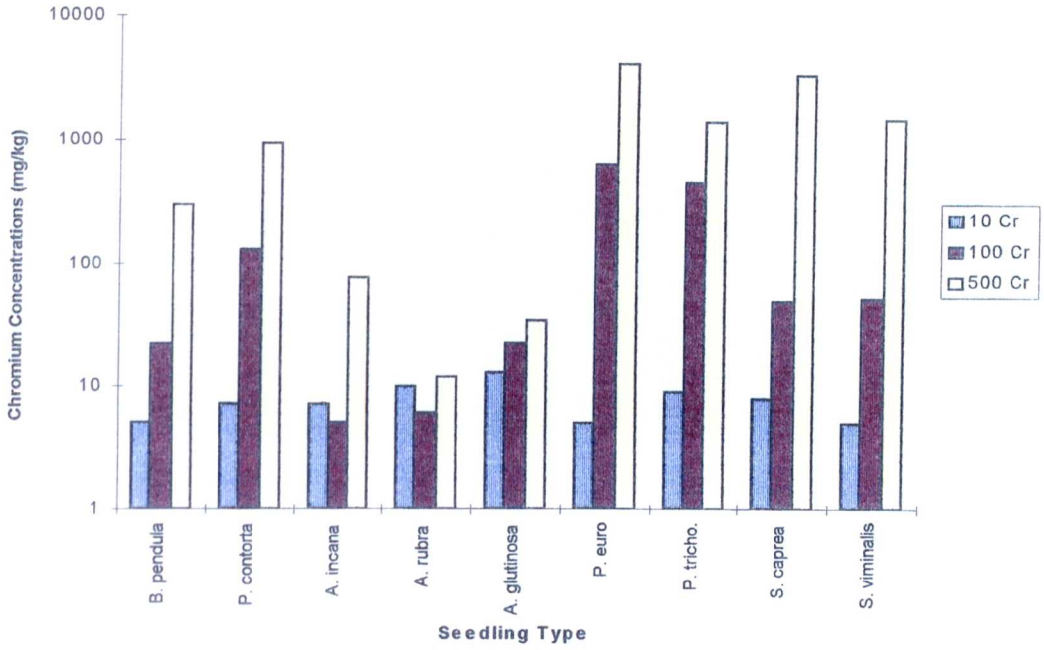


Figure 5.2 Maximum Metal Concentrations in Foliage (10 mg/l Nutrient Solution)

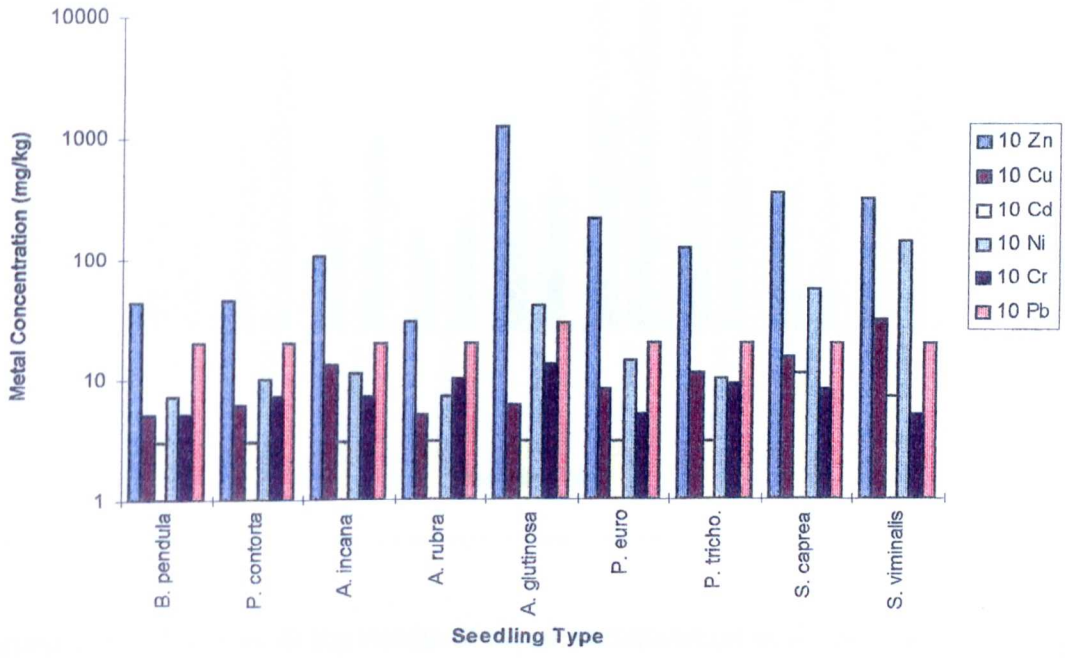


Figure 5.3 Maximum Metal Concentrations in Foliage (100 mg/l Nutrient Solution)

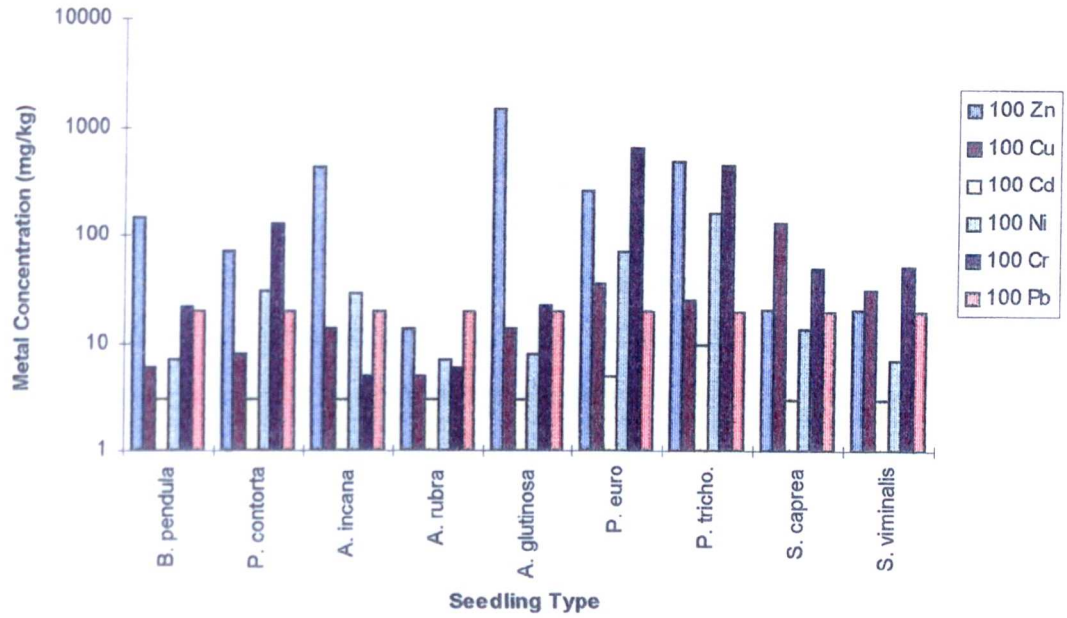
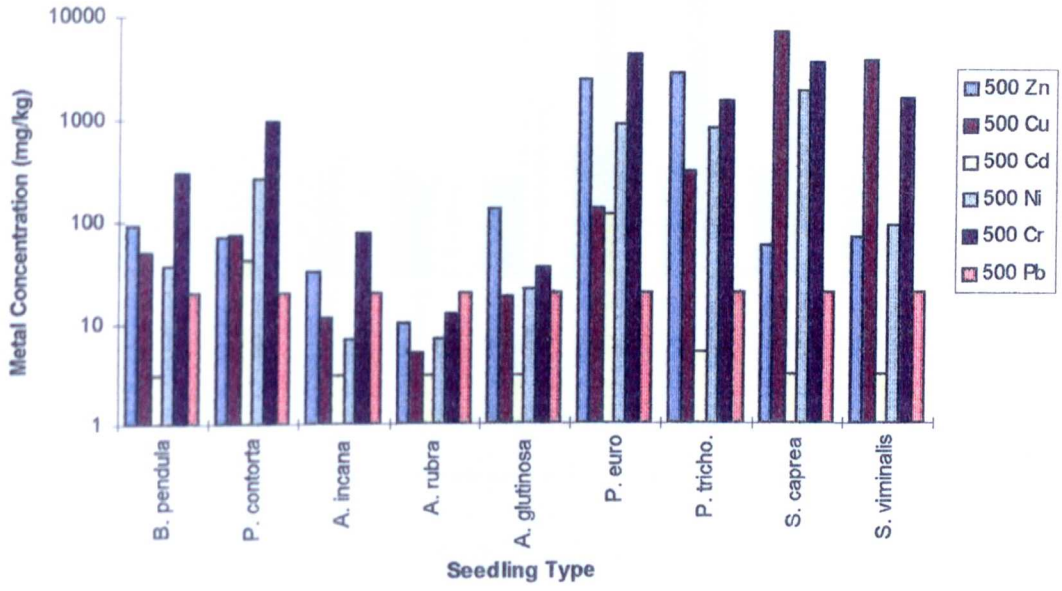


Figure 5.4 Maximum Metal Concentrations in Foliage (500 mg/l Nutrient Solution)



Figures 5.1 to 5.4 present the results of metal accumulation in foliage (leaves). Figure 5.1 'Chromium Concentrations in Foliage' shows the concentrations of chromium analysed in foliage as a function of the chromium concentration in nutrient solution.

In most of the study seedlings except *A. incana* and *A. rubra* an increase in chromium solution concentration has resulted in an increasing level of chromium in foliage. Both *Populus* and *Salix* species accumulated higher concentrations of chromium compared to the other species.

Figures 5.2 to 5.4 'Maximum Metal Concentrations in Foliage' for solution treatments of 10, 100 and 500 mg l^{-1} respectively. At low solution concentrations (10 mg l^{-1}) the general order of metal accumulation from highest to lowest was Zn > Pb, Ni >Cr, Cu > Cd. The results are influenced by the limits of quantification given. At higher concentrations (500 mg l^{-1}) the order of accumulation was Cr, Zn > Cu, Ni > Pb > Cd.

Figure 5.5 Copper Concentrations in Twigs

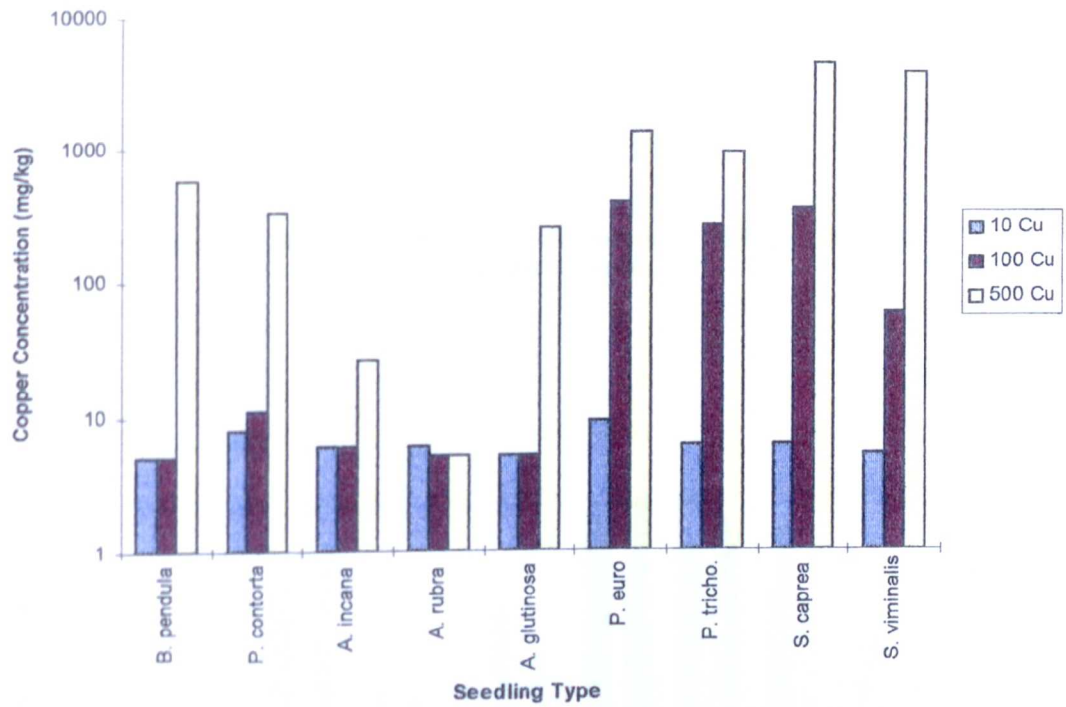


Figure 5.6 Maximum Metal Concentrations in Foliage (10 mg/l Nutrient Solutions)

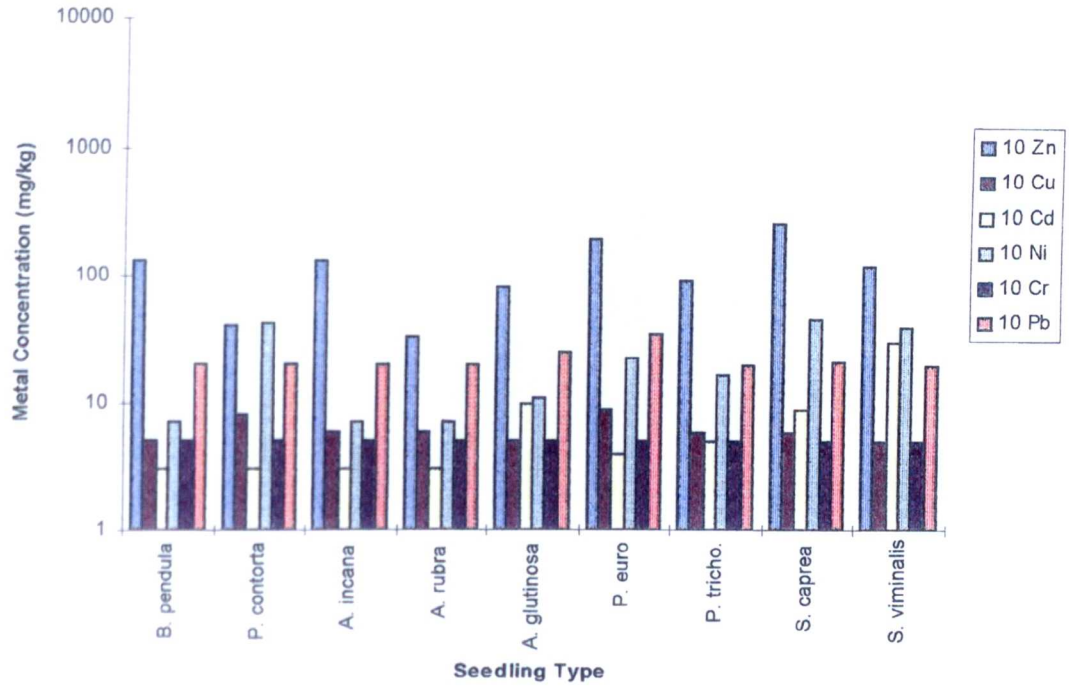


Figure 5.7 Maximum Metal Concentrations in Foliage (100 mg/l Nutrient Solution)

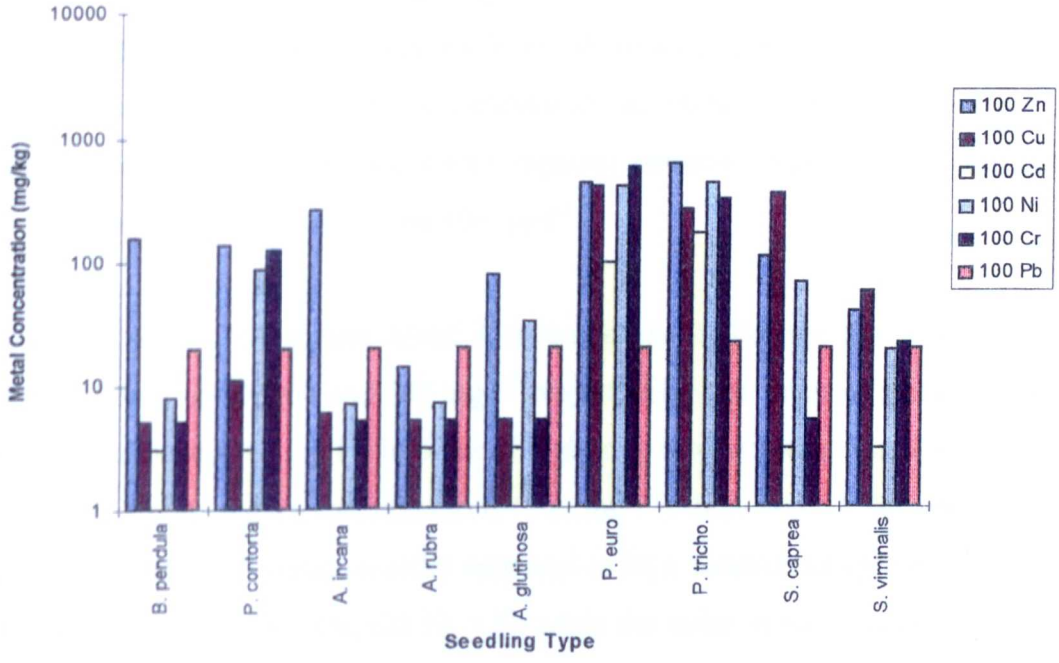
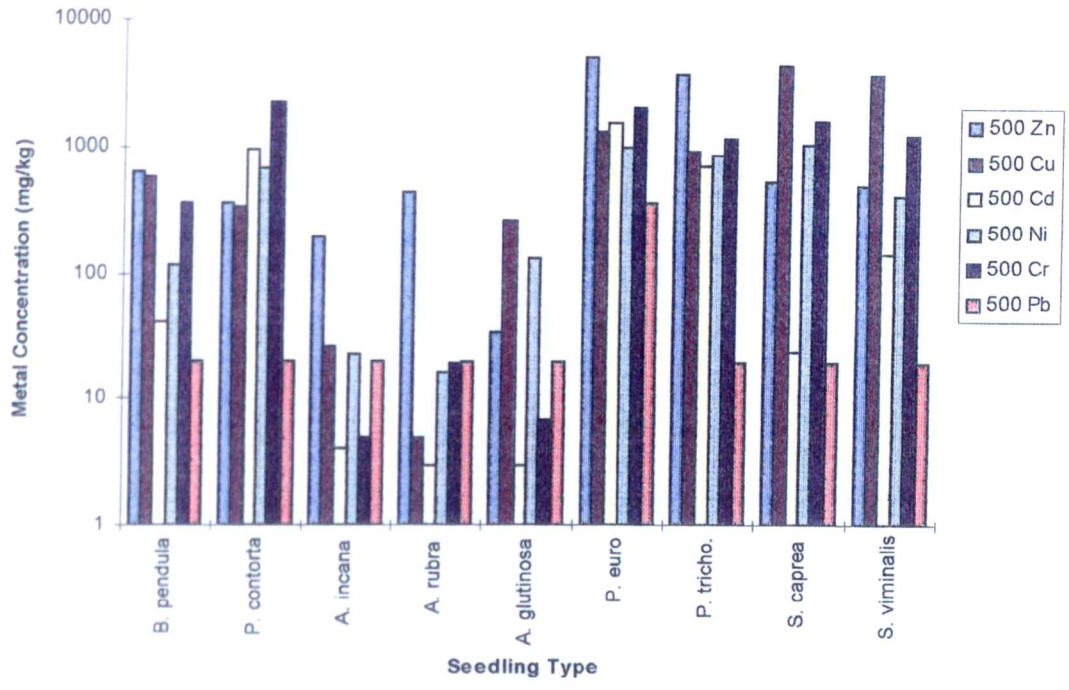


Figure 5.8 Maximum Metal Concentrations in Twigs (500 mg/l Nutrient Solutions)



Figures 5.5 to 5.8 present the results of metal accumulation in Foliage (Twigs). Figure 5.5 'Copper Concentrations in Twigs' has been included to show the accumulation of copper in twigs as a function of the copper concentration in nutrient solution. In both *Populus* and *Salix* seedlings, increasing levels of copper accumulation was recorded in response to increasing copper concentrations in nutrient solution, however no difference in copper accumulation was apparent between *Betula, Pinus* and *Alnus* species at treatment levels of 10 and 100 mg l^{-1}.

Figures 5.6 to 5.8 'Maximum Metal Concentrations in Foliage (Twigs)' at metal treatment levels of 10, 100 and 500 mg l^{-1} respectively. The order of metal uptake at low treatment levels (10 mg l^{-1}) in order of highest to lowest uptake was Zn > Pb >Ni, Cd, Cu and Cr. At higher concentrations (500 mg l^{-1}) there was no definite order of accumulation and accumulation order appeared to be a function of species. The order in *Populus* was Zn > Cr > Cu, Cd, Ni > Pb while the order in *Salix* was Cu > Cr, Ni, Zn > Cd > Pb.

Figure 5.9 Nickel and Lead in Roots

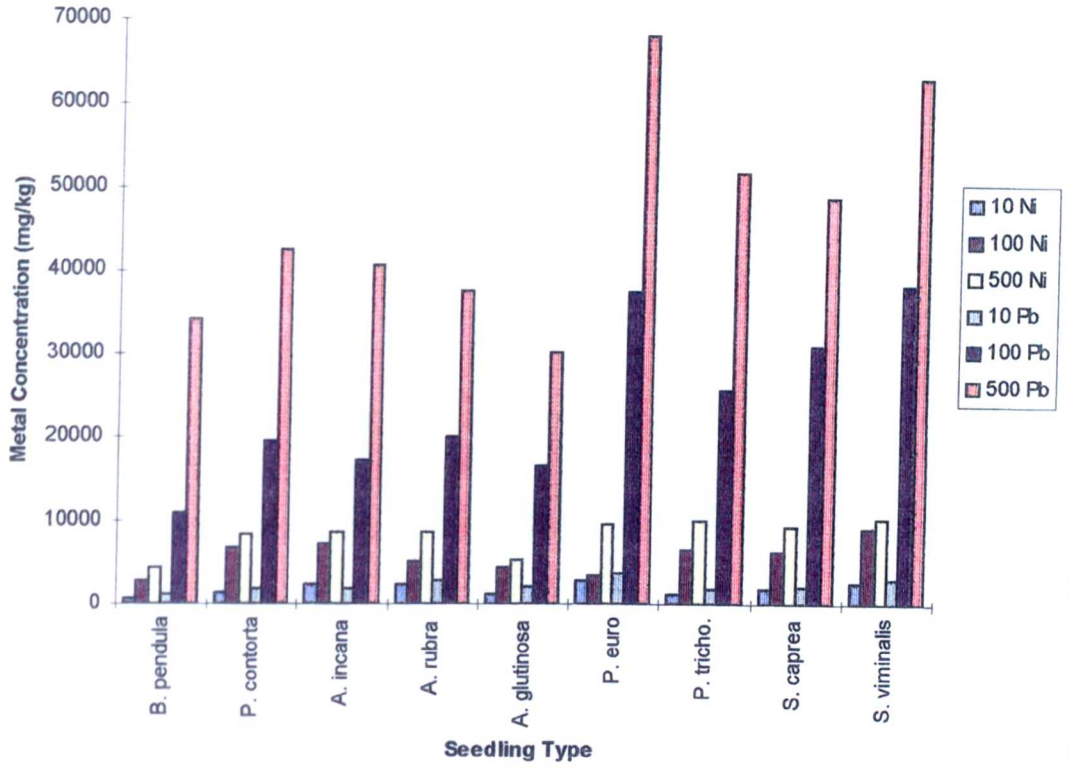


Figure 5.10 Maximum Metal Concentrations in Roots (10 mg/l Nutrient Solutions)

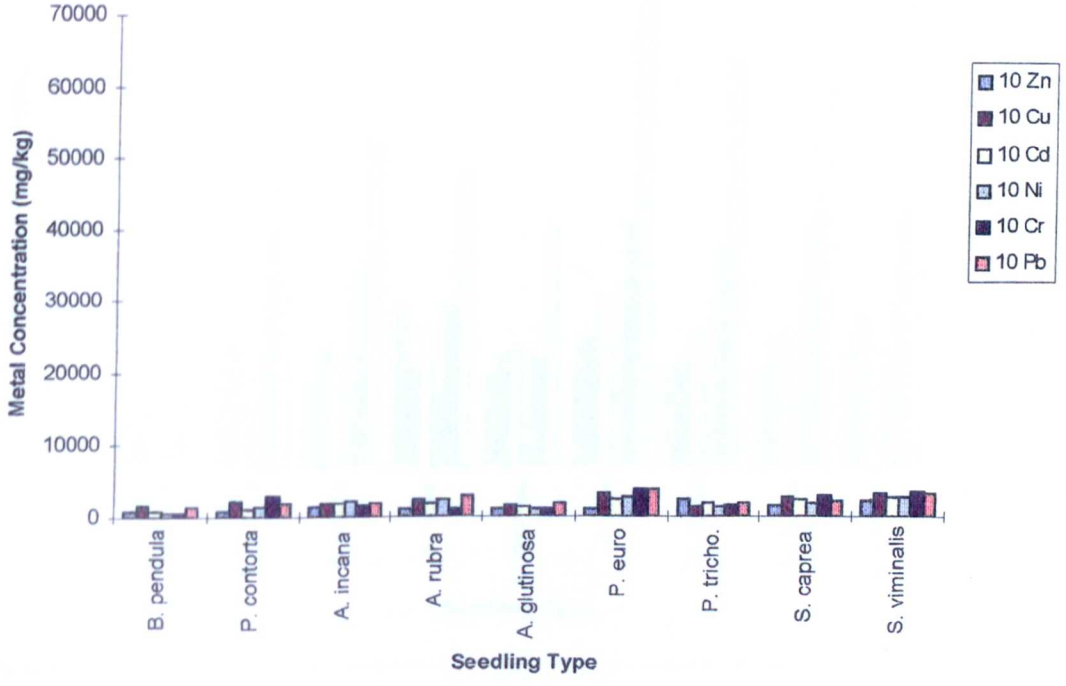


Figure 5.11 Metal Concentrations in Roots (100 mg/l Nutient Solutions)

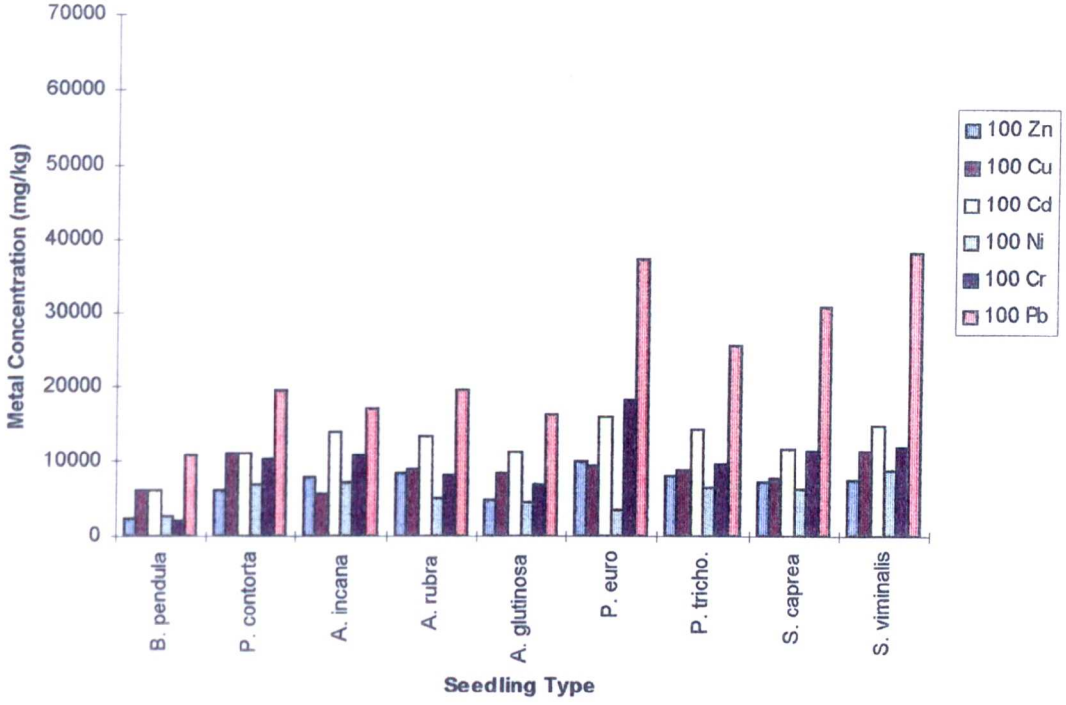
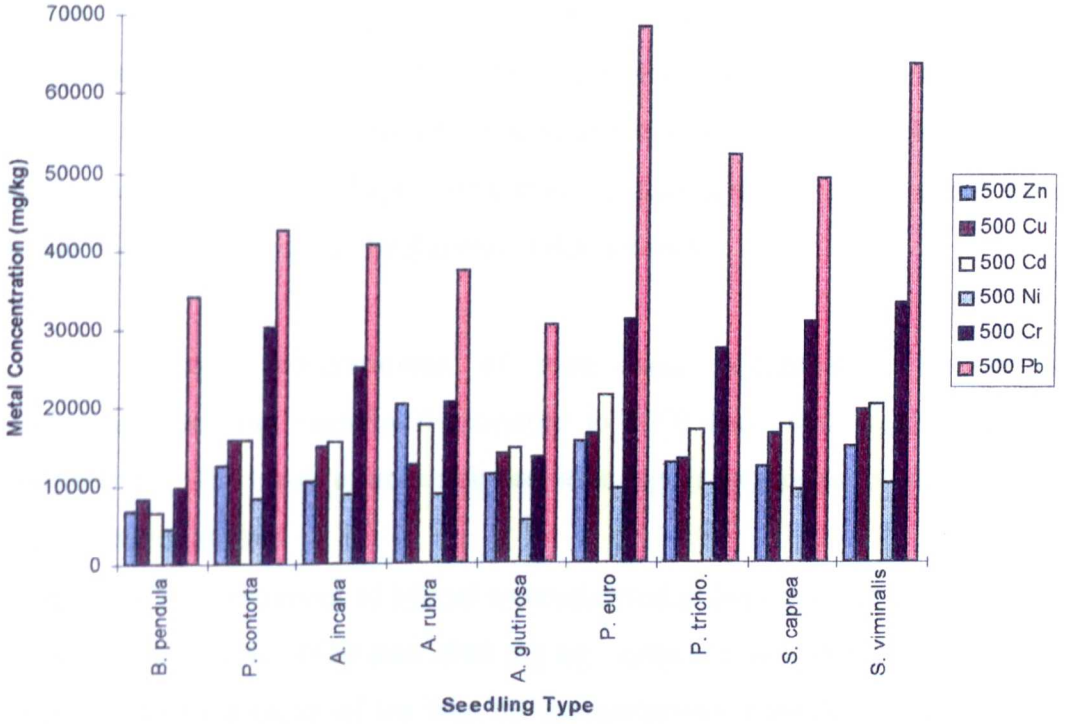


Figure 5.12 Metal Concentrations in Roots (500 mg/l Nutrient Solutions)



Figures 5.9 to 5.12 present the results of metal uptake/accumulation in the roots of the study seedlings. Figure 5.9 'Nickel and Lead in Roots' has been included to show the level of nickel and lead accumulation in roots as a function of the external metal concentration in nutrient solution. Increasing levels of metal accumulation have been recorded with increasing lead concentrations in solution. The figure also shows the variation in uptake between the two different study metals.

Figures 5.10 to 5.12 'Maximum Metal Concentrations in Roots' at metal treatment levels of 10, 100 and 500 mg l^{-1} respectively. The order of metal accumulation at low treatment levels (10 mg l^{-1}) in the order of highest to lowest accumulation was generally Cu, Cr, Pb > Cd, Ni > Zn. At higher treatment levels (500 mg l^{-1}) the order was Pb > Cr > Cu, Cd > Zn, Ni.

5.2.4 Discussion

The analysis of the biomass samples recovered from the study seedlings grown in the nutrient solutions generally show that the uptake of each metal increases with increasing solution concentration. These increased levels of metal uptake were recorded in leaves, stems, twigs and roots. For all metal treatments the highest metal accumulation was in the root samples. It was also noticeable from the results that one or two tree species in each treatment showed above average accumulation of specific metals to twigs, stem and foliage. The uptake of studied metals to the above ground biomass of the study trees can be summarised as follows:

- above average concentrations of zinc were accumulated by *Populus euroamericana* and *Populus trichocarpa* to 5250 and 3860 mg kg^{-1} in twigs respectively. The levels of accumulation were higher by a factor of ten than in the other species studied
- significant concentrations of copper were analysed in twig samples of *Salix caprea* and *Salix viminalis* (4530 and 3840 mg kg^{-1} respectively). These concentrations were higher by a factor of ten than the concentrations recorded in the other tree species studied.

- the highest accumulation of cadmium was by *Populus euroamericana, Populus trichocarpa* and *Pinus contorta.* The levels in respective twig samples were 1590, 737 and 951 mg kg^{-1}.
- the highest concentrations of nickel were recorded in the twig and leaf samples of *Populus euroamericana, Populus trichocarpa, Salix caprea* and *Pinus contorta.*
- accumulation of chromium was recorded in the foliage and twigs of *Populus euroamericana, Populus trichocarpa, Salix caprea, Salix viminalis* and *Pinus contorta.*
- uptake of lead was only noted at the highest solution concentration in *Populus euroamericana* (364 mg kg^{-1}).

The results obtained from the hydroponic studies demonstrate that some trees preferentially accumulate some metals. For all study metals the highest accumulation rates were generally by either poplar or willow. Given that these species were the highest yielding species in this study, the potential of these species to be used for phytoremediation is high, provided the correct species is selected for the nature of contamination found. The results, especially for lead (364 mg kg^{-1}) indicate that lead accumulation in *Populus euroamericana* is significantly lower than the concentration of 10,300 mg kg^{-1} recorded by Kumar et al. (1995) in *Brassica juncea* at similar treatment levels.

5.3 TREE SEEDLING HEALTH

5.3.1 Introduction

Satisfactory tree health and growth depends on the availability of essential nutrients in the soil. Should these essential nutrients be unavailable some visual deficiency symptoms can often be observed. These deficiency symptoms can include poor growth rate, reduction in needle or leaf size, yellowing of foliage, premature senescence or leaf loss. Similar effects can be caused by the presence of toxic metals (Weier et al., 1982). In this regard during the course of the experiment and prior to the collection of

foliage samples a visual assessment was made on the health of the respective seedlings within each treatment.

5.3.2 Results

The short notes made on the health of the tree seedlings within each treatment have been reproduced in Table 5.7 which is included in Appendix 4.

5.3.3 Discussion

The visual assessment of the tree seedlings during the experiment shows that with most treatments the added metals had a detrimental effect on growth. This was particularly evident at the higher metal concentrations when most of the foliage wilted and died. The experiment was run for a relatively short period of time but by the end of the experiment fresh growth was noted on a number of seedlings. These findings are considered to indicate that the initial shock of the metal was toxic to the seedlings and the water uptake was significantly altered. However with time the trees became more tolerant and started to recover. This recovery could be attributed to removal of free metal from the solution or tolerance adaptations by the individual seedlings.

5.4 SELECTIVE EXTRACTION OF SEEDLING ROOTS

5.4.1 Introduction

When plant roots are immersed in nutrient solutions containing elevated concentrations of heavy metals some of the metals will be taken up by the plant roots. The uptake of heavy metals into the plant requires the transfer of metal across the root surface and cell membranes (Leavitt et al., 1979). However, it is likely that some metal will bind to the external surface of the root. As with metals in the soil the strength of binding will be dependent on the metal, and selective extractants can be used to extract different pools of metal (Sheppard & Thibault, 1992).

This experiment considers the use of selective extractants, originally developed for extracting metals from soil, to extract root bound metals (Khattack, 1987). Ammonium acetate was chosen to remove surface adsorbed and weakly bound metal, ammonium EDTA was chosen to extract strongly held external metal and the concentration of internal residual metal was assessed by acid digestion.

5.4.2 Procedure

The root samples recovered at final harvest from the hydroponic solutions described previously were not analysed by selective extraction procedures. The results discussed in this Section only relate to a study experiment undertaken by an undergraduate student, Udo Schroeter (unpublished work) which set out to study the binding and uptake of metals by plant roots.

Willow (*Salix viminalis*) and poplar (*Populus trichocarpa*) seedlings were established in hydroponic solutions in a similar manner to those discussed in Section 2.7. Zinc, chromium and lead were added to the nutrient solutions to give metal concentrations of 1000 mg l^{-1}. Duplicate trees of each species and metal treatments were harvested at weekly intervals over a four week period and the root samples were analysed by sequential selective extraction procedures in accordance with documented methods (Section 2.4.2).

5.4.3 Results

The concentration of each metal extracted by each extractant is given in Table 5.8a (Poplar) and 5.8b (Willow). The selective extraction results have also been expressed as a percentage of the total metal removed by each treatment in Table 5.9. These results assume that the metal removed by ammonium EDTA would have included the fraction removed by calcium chloride had the procedure been non-sequential. The total metal values are the sum of all metal concentrations extracted by the three procedures.

The values given are means of duplicate analysis results, the standard deviation of these results is expressed as the ± values.

Table 5.8a Selective Extraction of *Populus trichocarpa* Roots

Tree Species	Study Metal	Extractant	Concentration of Measured Metal mg kg^{-1} dry weight			
			Week 1	Week 2	Week 3	Week 4
Poplar	Zinc	Ammonium Acetate	1246 ± 570	2000 ± 580	8110 ± 1260	14200 ± 3350
		Ammonium EDTA	5780 ± 1250	11300 ± 3290	32400 ± 5500	41500 ± 13700
		Residual	4000 ± 570	7690 ± 2230	10100 ± 1030	10900 ± 910
		Total	11026	20990	50610	66600
	Chromium	Ammonium Acetate	560 ± 160	690 ± 270	4270 ± 2590	4300 ± 1390
		Ammonium EDTA	130 ± 25	106 ± 40	1820 ± 1030	2210 ± 370
		Residual	16500 ± 1120	21900 ± 3640	26700 ± 5790	32000 ± 2800
		Total	17190	22690	32790	38510
	Lead	Ammonium Acetate	910 ± 910	1070 ± 330	9980 ± 7620	5020 ± 5250
		Ammonium EDTA	14900 ± 3070	19200 ± 2820	94700 ± 69000	77800 ± 46800
		Residual	15900 ± 4600	24600 ± 5800	31500 ± 6690	29600 ± 12500
		Total	31710	44870	136180	112420

Table 5.8b Selective Extraction of *Salix viminalis* Roots

Tree Species	Study Metal	Extractant	Concentration of Measured Metal mg kg^{-1} dry weight			
			Week 1	Week 2	Week 3	Week 4
Willow	Zinc	Ammonium Acetate	1370 ± 270	2870 ± 1880	26400 ± 22300	12760 ± 6300
		Ammonium EDTA	5910 ± 1470	10000 ± 4490	43800 ± 16600	29900 ± 14000
		Residual	3500 ± 530	8070 ± 3400	10800 ± 2500	10300 ± 2500
		Total	10780	20940	81000	52960
	Chromium	Ammonium Acetate	340 ± 55	640 ± 160	2400 ± 1100	1480 ± 410
		Ammonium EDTA	70 ± 10	60 ± 30	800 ± 230	690 ± 210
		Residual	12900 ± 2520	17600 ± 2100	23600 ± 2900	28800 ± 3300
		Total	13310	18300	26800	30970
	Lead	Ammonium Acetate	890 ± 220	1300 ± 200	2920 ± 1670	4260 ± 1170
		Ammonium EDTA	10800 ± 1270	17800 ± 2460	80100 ± 24900	86500 ±18300
		Residual	8980 ± 1420	18800 ± 4400	25500 ± 5210	29500 ± 3600
		Total	20670	37900	108520	120260

Table 5.9 Percentage of Total Metal Extracted by Ammonium Acetate and Ammonium EDTA

Metal	Week	*Populus trichocarpa*		*Salix viminalis*	
		Ammonium Acetate	Ammonium EDTA	Ammonium Acetate	Ammonium EDTA
Zinc	1	11.3	63.7	12.7	67.5
	2	9.5	63.3	13.7	61.5
	3	16.0	80	32.6	87.0
	4	21.3	83.6	24.0	80.6
Chromium	1	3.3	4.0	2.6	3.1
	2	3.0	3.5	3.5	3.8
	3	13	18.6	9.0	11.9
	4	11.2	16.9	4.8	7.0
Lead	1	2.9	49.9	4.3	57.0
	2	2.4	45.2	3.4	50.4
	3	7.3	77.0	2.7	76.5
	4	4.5	73.7	3.5	75.0

5.4.4 Discussion

The total uptake of each metal by the study roots shows an overall increase over the four week period of the study except for a couple of samples in week 3 which has been attributed to analytical error. The order of metal accumulation starting with the highest accumulation was Pb > Zn > Cr during the study period. This order differs from the findings reported in Section 5.2 where the order of metal accumulation in the seedling roots was Pb > Cr > Zn.

The percentage of total root metal removed by ammonium acetate from poplar and willow roots over the four week period varied between 9.5 and 32 % zinc, 2.6 and 7.3 % chromium and 2.4 and 7.3 % lead. An increasing percentage of metal was removed by ammonium acetate with time. The relatively low percentages of metals removed by ammonium acetate would tend to indicate that the study metals are either held strongly on the outer surface of the root or have passed beyond the outer membrane of the root.

The percentage of total metal removed by ammonium EDTA from poplar and willow roots was higher than the percentage removed by ammonium acetate and varied between 61.5 and 87 % zinc, 3.1 and 18.6 % chromium and 45.2 and 76 % lead. These results show that a quantity of zinc and lead was bound on the external surface of the roots. The strength of binding was such that the metal was not removed by ammonium acetate. The lower percentage of extractable chromium may be due to either the chromium being present as the chromate anion and not being extracted by ammonium EDTA or most of the chromium being held internally within the root.

These studies can give an indication of which metals bind to the root surface and which metals are taken up across cell membranes. When such studies are compared with metal uptake studies it is often found that metals which accumulate in the root are not accumulated in foliage.

5.5 SOLUTION SALINITY and pH

5.5.1 Introduction

As previously discussed the pH at which metal uptake studies are undertaken is an important factor which can influence the availability of metals to vegetation. The effect of pH is likely to be less pronounced in nutrient solution studies primarily due to the omission of the soil factor and the impact on the cation exchange capacity of the soil. As previously discussed in Section 4.2.2 soil salinity can be toxic to vegetation by creating conditions which can restrict the uptake of water and nutrients.

5.5.2 Procedure

The pH and salinity of the nutrient solutions (6 metals at solution concentrations of 10, 100 and 500 mg l^{-1}) were measured in samples of solution recovered from the experiment after final harvesting of seedlings (Section 5.2). The pH and salinity were

measured directly in the respective solutions using a calibrated pH meter and conductivity meter (Refer Section 2.3.1 and 2.3.2).

5.5.3 Results

The recorded pH and salinity values are tabulated in Table 5.10 'Solution pH and Salinity'. The salinity values have been given a Conductivity Index in accordance with guidance provided by MAFF, 1988 (Refer Table 2.1).

Table 5.10. Hydroponic Solution pH and Salinity

Metal	Intended Solution Concentration mg l^{-1}	Solution pH	Solution Salinity µS cm^{-1}	Conductivity Index
Zinc	10	6.4	65	0
	100	5.9	372	1
	500	5.8	1730	7
Copper	10	4.9	105	0
	100	4.2	394	1
	500	4.7	1470	7
Nickel	10	6.9	120	0
	100	4.6	504	1
	500	6.2	1770	7
Cadmium	10	6.7	69	0
	100	6.4	291	0
	500	5.4	1040	5
Chromium	10	6.8	62	0
	100	7.3	361	1
	500	6.3	1400	6
Lead	10	6.9	24	0
	100	7.0	36	0
	500	5.2	526	2
Control		6.5	198	0

5.5.4 Discussion

The salinity of each nutrient solution increased with the addition of more inorganic metal salt as would be expected. The Conductivity Index value and the severity of salinity suggested by MAFF (1988) is conductivity index 0-2 (no growth restrictions should apply), 2-4 (possible growth restrictions may be experienced in young plants) and >4 (severe crop damage is likely). In this regard all 10 and 100 mg l^{-1} metal treatments had conductivity index values less than 2 and therefore no growth restrictions should be caused by solution salinity. All 500 mg l^{-1} treatments except Pb had conductivity indexes in excess of 4 indicating the toxicity problems may have been due to solution salinity.

The order of salinity at each respective solution concentration starting with the highest was nickel > copper > cadmium > zinc > chromium > lead.

The solution pH values were variable and no clear trends were evident between metal treatments. In most treatments the pH of the solutions were changed by the addition of metal salts. It is not known whether it was the addition of the metal that changed the solution pH or whether it was the action of the added metals on the tree roots which caused the release of H^+ ions into solution. The results indicate that in the majority of treatments the added metals caused an increase in solution acidity.

5.6 FINAL METAL CONCENTRATIONS IN NUTRIENT SOLUTION

5.6.1 Introduction

On completion of the hydroponic studies representative samples of the remaining nutrient solutions were collected for total metal analysis in order to determine final metal concentrations. It should be noted at the time of sampling no accurate measurement was made of the nutrient solution volume and therefore the weight of metal removed from each treatment cannot be estimated. The results are included as general information.

5.6.2 Procedure

The nutrient solutions were analysed by atomic absorption spectrometry in accordance with documented procedures, refer Section 2.5.

5.6.3 Results

The final metal concentrations are tabulated in Table 5.11.

Table 5.11 Final Metal Concentrations in Nutrient Solution

Metal	Starting Solution concentration mg l^{-1}	Actual weight Metal (g)	Measured Solution Concentration mg l^{-1}
Zinc	10.2 101 500.9	0.1636 1.613 8.002	1.35 63.1 462
Copper	10.5 103.4 504.4	0.1677 1.655 8.072	0.73 51.4 354
Cadmium	10.6 100.2 502	0.1693 1.595 7.965	1.2 40 411
Nickel	10.3 100 501	0.1645 1.601 8.014	3.67 107.8 475
Chromium	10.4 100 501	0.1664 1.602 8.003	1.0 30 245
Lead	10.5 101 502	0.1686 1.6232 8.052	0.2 1.0 137

5.6.4 Discussion

The removal of metal from the nutrient solutions has been assumed to be by a process of plant uptake and adsorption. However, it is also likely that some metal will have been precipitated in some of the metal treatments from solution. The final metal

concentrations recorded can be used to give a general indication of the order of metal removal by the seedlings. The order of metal removal starting with the highest level of removal to lowest was Pb > Cr > Cu > Cd > Zn > Ni. These findings are very similar to the order of metal accumulation in roots recorded in Section 5.2 of Pb > Cr > Cu, Cd >Zn > Ni.

5.7 SUMMARY

The hydroponic experiments have shown the potential of some trees to accumulate heavy metals in roots and above ground tissues from nutrient solutions. It may even be suggested from the results that trees can hyperaccumulate metals, particularly in roots The levels of lead accumulation in root and foliage are lower than accumulation levels reported by Kumar et al. (1995) in *Brassica juncea* for similar metal treatment concentrations, however trees have other benefits including the production of a commercial crop.

The hydroponic metal nutrient solutions, excluding the 500 mg l^{-1} treatments, had lower salinity levels than the pot soil treatments and were therefore likely to be of less stress to the seedling in respect of salinity. Therefore toxic symptoms could be attributed to metal toxicity and not water stress.

The use of hydroponics could be used as a screening procedure for determining metal accumulation traits in tree seedlings. The results also show that tree roots are very good at binding and removing heavy metals. In this regard trees planted in contaminated soil are likely to scavenge and retain metals present in the soil solution; this could be beneficial in reducing the leaching and subsequent migration of metal contaminants from polluted sites.

Both poplar and willow species showed an ability to accumulate a number of the studied heavy metals to concentrations higher than other study tree species. Given that both species are some of the highest yielding coppice species and that plant breeding

trials are producing higher yielding clones, the potential for cleaning up contaminated sites could be extensive.

The hydroponic studies will be further discussed in Chapter 6.

CHAPTER 6
GENERAL DISCUSSION AND CONCLUSIONS

Throughout the United Kingdom there are many thousands of hectares of contaminated and derelict land which require remedial works prior to redevelopment. Records published by the Department of The Environment (1991a) and Scottish Office (1990) estimate that there is approximately 40,500 hectares of such land in England and Wales and 7400 hectares in Scotland which fall within this category. The investigation and remediation of such sites can be very expensive and as a result some sites are deemed to be so contaminated that no redevelopment will occur without government funding. The problem is not confined to the UK but is a problem experienced by most countries. Cunningham & Berti (1993) suggest that the United States spends billions of dollars each year on the remediation of contaminated soils. When remedial works are undertaken it is common practice for contaminated soil to be excavated and removed from site to a suitable landfill facility. Most often the remedial works make use of engineering based technologies which can be grouped in to two broad categories: a) isolation and containment, and b) decontamination.

Whilst heavy metals in the soil cannot be destroyed, they can be changed to less toxic chemical forms but for this to happen in-situ chemical reactions are required. In an attempt to investigate alternative methods of cleaning up soil contaminated by heavy metals, this project was devised to assess the feasibility of removing heavy metals from site by the process of 'phytoremediation'. Phytoremediation harnesses the ability of plants to accumulate heavy metals from the soil within their tissues so that when the plant is harvested the metal held within the plant is also removed from the site. All plants are known to take up nutrients required for growth from the soil solution, however little information is available on the uptake of heavy metals especially by trees. Plants which colonise mine waste and contaminated soil can accumulate very high concentrations of heavy metals within their tissues and are often referred to as hyperaccumulators (Kumar et al., 1995).

The process of phytoremediation and hyperaccumulation of metals by selected vegetation has been reported by a number of authors. Studies have found that certain plants can concentrate essential and non-essential heavy metals in their roots and shoots to levels far

exceeding those present in the soil. Such metal accumulating plants are often found growing on metalliferous soils found in different regions around the world (Raskin et al., 1994) and Brown et al. (1994 and 1995) evaluated the potential of phytoremediation with respect to two metallophytes, *Thlaspi caerulescens* (Zn accumulator) and *Silene vulgaris* (Zn indicator). *Thlaspi caerulescens* showed much greater tolerance to cadmium and zinc and accumulated up to 18455 mg kg^{-1} Zn and 1020 mg kg^{-1} Cd per dry weight of shoots.

Some crop Brassicas have been shown to accumulate heavy metals in roots and shoots, particularly from nutrient solution. Kumar et al. (1995) and Dushenkov et al. (1995) reported that *Brassica juncea* (L.) Czern (Indian Mustard) removed toxic metals (Cu^{2+}, Cd^{2+}, Cr^{6+}, Ni^{2+}, Pb^{2+} and Zn^{2+}) from aqueous solutions and measured lead concentrations in roots and shoots of 103,500 and 10,300 mg kg^{-1} dry weight respectively.

In selecting a plant species for heavy metal extraction, the ability to accumulate high concentrations of heavy metals is but one of the traits which must be considered. Cunningham & Berti (1993) identified the following as attributes desirable in plants to be used in phytoremediation:

- the selected plants should have the ability to accumulate and retain high concentrations of one or more heavy metals in the above ground biomass
- high yielding crops should be used where possible
- the selected plants should have an extensive root network to increase the depth and volume of soil which can be remediated
- the selected plants should be easily harvested by mechanical means
- added benefits would be gained if the crop did not have to be replanted after harvest and the harvested crop had an economic value

With reference to the desirable attributes above, coppice woodland meets most of the criteria.

Interest in coppice woodland has been re-established due to the increasing interest in using the timber produced as fuel to generate electricity. Due to increasing environmental concerns over the use of fossil fuels (gas, coal, oil) and the finite nature of these resources, alternative renewable energy sources are being sought. Trials have been undertaken on the use of wood as a fuel for energy generation and recent breeding and growth trials on willow and poplar hybrid species have shown that willow hybrids yield on average 18 t ha^{-1} y^{-1} (60 green t ha^{-1} y^{-1}, Deboys, 1994). On this basis 400 - 600 hectares of coppice would be required for the continual generation of 1MW of electricity (Piper, 1994). Establishment cost may be in the order of £2350.00/hectare and once planted the crop can be harvested on average 10 times before replanting and the harvested crop can be sold for in the order of £20 to £25/t. Forestry Authority grants are also available (£400-600/hectare) for planting short rotation coppice (Pile, 1995).

In addition to their potential economic return for power generation it is also worth noting that trees have been and are still planted on contaminated land as part of site remediation works to screen the site, provide a physical barrier to restrict access and to stabilise the soil and prevent erosion.

This research project comprised various works to determine the uptake and accumulation of heavy metals by different species of tree including willow and poplar hybrids. The experimental works were undertaken as a feasibility exercise to assess the accumulation of heavy metals by woodland species grown in both soil and nutrient solution environments and the findings have been reported in Chapters 3 to 5. The works comprised three main studies which have been referred to as 'field', 'pot' and 'hydroponic' studies. The three experiments were designed to be complementary to each other in that they had one or more comparable tree species within each treatment and metal treatment levels were comparable between soil and nutrient solution. The main difference between the experiments was primarily the availability to the seedlings of the applied heavy metals. The availability of metal has been reported to influence the plant uptake of cadmium. Haghiri (1974) reported that the cation exchange capacity, organic

matter content, zinc concentration and temperature of the soil all influenced the uptake of cadmium.

Field Studies

The field studies set out to monitor metal accumulation rates in naturally established forests growing on heavy metal contaminated soil and involved the collection and analysis of biomass samples from trees growing on contaminated soil. Two different sites which were contaminated by various heavy metals were utilised for the study.

The sites were identified as Summerford and Redding and analysis of soils from each site confirmed that the soils at both sites were contaminated with heavy metals. Summerford was the site of a former chrome works and chromium waste had been tipped on site, as a result the soils analysed contained elevated concentrations of chromium to a maximum concentration of 1700 mg kg^{-1} and to a lesser extent nickel. Redding was a former explosives factory manufacturing detonators, as a result the soil at the site was contaminated with copper, nickel, mercury, zinc and lead. The maximum concentrations of heavy metals detected at Redding was 2580 mg kg^{-1} zinc, 79100 mg kg^{-1} copper and 18600 mg kg^{-1} lead. The level of metal concentrations and soil contamination at each site were generally lower than ground contamination levels reported in the vicinity of smelters and within metalliferous mine spoil (29,000 mg kg^{-1} lead, 20,000 mg kg^{-1} copper, 12,000 mg kg^{-1} nickel and 10,000 mg kg^{-1} zinc were reported by Hogan & Wotton, (1984), Hazlett et al., (1983), Tyler, (1984), Smith & Bradshaw, (1979) and Eltrop et al., (1991).

The distribution of heavy metals in the soil at Redding was very random and appeared to be confined to localised pockets, some of the soil material recovered containing high concentrations of plant available copper, lead and zinc. The soils at Summerford were generally more uniform and contained lower levels of plant available metals.

The results of the study demonstrated that the uptake of metals by trees growing on such 'field' sites was generally low. Zinc was accumulated to the highest levels, particularly in

the bark, twigs and leaves of study trees. In fact the level of zinc accumulation in twigs and leaves was often higher than the levels recorded in the soil at the site. The uptake of zinc by the study trees was considered to be a combination of site factors (metal concentration and availability) and of tree species. From the selective extraction assessment of heavy metal availability the highest concentrations of available metals were recorded at Redding. Uptake levels were higher in willow and birch growing at Redding compared to birch growing at Summerford, however, the sycamore growing at Redding had the lowest level of zinc uptake. At Redding the highest levels of zinc accumulation were not associated with areas of highest soil zinc concentrations. One explanation for this observation is that trees roots growing in contaminated soil may proliferate in the less contaminated areas of the soil so avoiding high metal concentrations (Turner & Dickinson, 1993). The general order of accumulation of the copper, zinc, lead and chromium in the foliage of the study trees starting with the highest accumulation was Zn > Cu, Pb > Cr.

The distribution of metal in above ground biomass in order of highest accumulation to lowest was leaves and bark > twigs > wood. Sander & Ericsson (1997) studied the vertical distribution of nutrients and heavy metals in *Salix viminalis* stems and reported that concentrations of Zn, Cu, Ni and Cd increased with height and was attributed to an increasing proportion of bark. The order of metal accumulation reported by Sander and Ericsson was 3-7 mg kg^{-1} chromium, 40-100 mg kg^{-1} zinc, 2-10 mg kg^{-1} copper, 1-6 mg kg^{-1} nickel and 0.7- 1.5 mg kg^{-1} cadmium. These levels are not dissimilar from the 'field' study experiments except for zinc which was accumulated to higher concentrations at the 'field' sites.

The highest levels of chromium, lead and copper were recorded in the roots of the study species and generally reflected the concentrations measured in soil. These results were confirmed by the hydroponic studies which showed that the highest percentage of chromium, lead and copper were retained in the roots.

The increasing concentrations of zinc recorded in the foliage of the study trees indicates that significant quantities of zinc will be returned to the soil at leaf fall. From the field study the highest concentrations of zinc in twigs, bark and wood occurred over the winter months. These findings are similar to results reported by Mejnartowicz (1986).

It is common practice to harvest coppice woodland during the winter months. From the studies undertaken, winter is also likely to be the preferred time with regard to maximizing the removal of copper and zinc which are at their highest concentrations in the bark and twigs. The harvest of coppice woodland just before leaf fall would be beneficial in reducing the level of zinc returned to the soil at leaf fall.

An autumn harvest would also coincide with the lowest moisture contents in the wood and since it has been suggested (Piper, 1994) that the efficiency of burning wood chips is linked to the moisture content an autumn harvest may be the optimum in terms of energy generation.

Any future 'field' experiments designed to study the accumulation of metals in tree foliage should preferably select sites with a uniform distribution of contamination in the soil. Soil sampling should be more intensive in the vicinity of study trees to give greater assessment of the level of soil contamination.

Pot Studies

The 'pot' experiment was devised to study the uptake and accumulation of heavy metals by trees from artificially contaminated soils. The tree seedlings were selected to represent common reclamation and coppice species used in the UK and included species of alder, pine, willow and poplar.

Soil metal concentrations were selected on the basis of information available in the literature. Smilde (1981) studied the growth of *Populus euroamericana*, *Avena sativa*, *Spinacia oleracea* and *Phaseolus vulgaris* in pots of sewage sludge (pH 6.7). Metal

concentrations were (in mg kg^{-1} substrate) 100, 200 and 300 Cd, 1000, 2000 and 3000 Cr, Pb and Zn, 500, 1000 and 1500 Cu and 250, 500 and 750 Ni added as acetates. These levels were not dissimilar from the metal concentrations used in the pot experiment (500 and 3000 mg kg^{-1} zinc, 500 and 2000 mg kg^{-1} copper, 500 and 1000 mg kg^{-1} nickel, 300 and 1000 mg kg^{-1} cadmium, 2000 mg kg^{-1} lead and chromium). The metal concentrations were less than the maximum values measured in the soils recovered from the field study sites.

The experiment suffered high mortality rates with most metal treatments. The initial assessment attributed the high fatality rate to soil metal concentrations. On further assessment the salinity of the soil solution was measured and the salinity values in the 'pot' soils were assessed with reference to guidance issued by MAFF (1988). It was found that the level of soil salinity was such that in most treatments the salinity levels would be toxic to most plants. The results indicated that poor seedling survival may be related to a possible combination of soil salinity and metal toxicity. It should be noted that during the literature review no reference was made to the control of salinity and in this regard no consideration was given to salinity in the design of the experiment.

The metal accumulation results obtained from the pot experiment showed higher levels of metal accumulation rates when compared with the results of the field studies. The maximum metal concentrations recorded in seedling foliage were 3756 mg kg^{-1} zinc in *Populus trichocarpa*, 259 mg kg^{-1} copper in *Populus trichocarpa*, 684 mg kg^{-1} nickel in *Alnus incana*, 453 mg kg^{-1} cadmium in *Salix viminalis* and 2274 mg kg^{-1} chromium in *Populus trichocarpa* (expressed on dry weight basis). No accumulation of lead was recorded. The maximum accumulation levels reported by Smilde (1981) in poplar leaves were 2232 mg kg^{-1} zinc, 359 mg kg^{-1} cadmium, 578 mg kg^{-1} copper and 380 mg kg^{-1} nickel which are not too dissimilar form the 'pot' experiment results obtained.

The accumulation of metal in the pot experiment was attributed to the increased availability of the metals within the pot soils. The availability of the added metals was assessed by selective extraction procedures and extraction of the soil solution. The

residual metal concentrations in the pot soils were found to be almost double the intended soil metal concentrations. This was attributed to all the added metal being held in the <2mm soil fraction which comprised only 50% of the total soil volume. Since this >2mm fraction is considered to contribute little to a soil's chemical properties it is likely that the metal concentrations experienced by the seedlings were higher than intended.

In this regard the concentrations of metal extracted by calcium chloride were much higher than the assessed soil solution metal concentrations. However, when the calcium chloride results were amended to reflect concentrations in the total soil volume the two procedures gave more closely comparable results. Some variations were noted in the concentrations of chromium and cadmium. The metal adsorption studies found that the order of metal adsorption from highest to lowest was Pb > Cu > Cd > Ni > Zn > Cr and the order of metal availability was almost the opposite with the exception of zinc and chromium (in order of highest availability) Zn > Cr > Ni > Cd > Cu > Pb. These results confirm the link between metal availability and metal adsorption.

When considering the design of similar 'pot' experiments more consideration should be given to the effect of soil salinity. It is likely that the choice of metal salt will have a direct bearing on the resulting soil salinity and where possible the chosen salt should give the lowest possible salinity. Selection of nitrate salts may have been beneficial in that the added anion would have been a fertiliser and therefore beneficial to the plant rather than toxic. Simple soil adsorption studies could be undertaken to determine the exact weight of metal which should be added to a soil to satisfy all the soil's adsorption capacity. This would prevent the addition of excess metal which would remain in soil solution. The selection of soils with higher cation exchange capacities would also allow the addition of higher concentrations of metal salts if so required.

Hydroponic Studies

A number of published metal uptake studies reported by various researchers have been conducted in nutrient solution. The main advantage of such studies is the omission of the

soil factor which is often considered a complication. This theory arises because of the unpredictable behaviour of metals added to soil. As previously discussed there are many different soil metal reactions which influence the availability and toxicity of metals to plants including adsorption, chemisorption, exchange, precipitation, complexation and chelation (Haghiri, 1974, Sheppard & Thibault, 1992 and Shuman, 1991).

During the course of this research the hydroponic solution experiments allowed the study of a greater number of metal treatments and tree species in a shorter period of time. No purpose built system was used and the system was self designed using available materials (Hershey, 1994). The chosen metal solution concentrations were influenced by previous work including hyperaccumulation studies undertaken by Kumar et al (1995) and by the metal concentrations used in the 'pot' experiment.

Higher levels of metal accumulation were recorded in the hydroponic studies when compared with the results of the field and pot experiments, particularly in the roots of the study seedlings. The maximum heavy metal uptake rates recorded in the 'hydroponic' study are compared with the heavy metal uptake by roots of *Brassica juncea* in the following table (Dushenkov et al., 1995).

Metal	'Dushenkov Results'		Hydroponic Results	
	Solution Conc mg l^{-1}	Uptake mg kg^{-1}	Solution Conc mg l^{-1}	Uptake mg kg^{-1}
Zn	100	13147	100	10100 (*P. euroamericana*)
Cd	2	268	10	2750 (*S. viminalis*)
Cr	4	716	10	3600 (*P. euroamericana*)
Ni	10	1080	10	2700 (*P. euroamericana*)
Cu	6	2943	10	3280 (*S. viminalis*)
Pb	2	1126	10	3650 (*P. euroamericana*)

The above results primarily allow the comparison between the uptake of zinc and nickel given the standard metal treatment levels. From the results obtained the accumulation

rates recorded in the 'hydroponic' study are not dissimilar from those reported by Dushenkov et al. (1995). The results highlight that trees can remove metals from nutrient solutions as effectively as some other metal accumulating plants and therefore trees could be used to remove heavy metals from aqueous streams (rhizofiltration). The results indicate that tree roots have a high affinity for free metal ions in solution. Therefore trees could be integrated into specially designed filter beds and used for cleaning wastewater arising from landfills, industry and domestic supplies.

Brown et al. (1995) studied the accumulation of cadmium and zinc in *Thlaspi caerulescens* (Zn and Cd hyperaccumulator). The maximum accumulation rates recorded in shoot tissue was 1290 mg kg^{-1} cadmium and 26,000 mg kg^{-1} zinc at solution treatment levels of 3160μM for each respective metal. The maximum cadmium and zinc uptake levels recorded from the hydroponic solutions (500 mg l^{-1}) was 1590 mg kg^{-1} cadmium and 5250 mg kg^{-1} zinc in the twigs of *P. euroamericana*. This shows that *P. euroamericana* can accumulate cadmium, but not zinc to higher concentrations than an identified hyperaccumulator.

The greatest disappointment in the studies undertaken is that the metal concentrations used in the hydroponic and pot studies had a severe effect on the health of the study species. It has been suggested that plants are either accumulators or excluders of heavy metals (Baker, 1987). Excluders are believed to have a control mechanism which restricts the uptake of heavy metals, however when this control mechanism is overloaded, metals flood into the plant. Given the ill effects shown by a number of study trees it is considered that the control mechanism of some of the study species may have failed. If this was the case it would be difficult to argue that the levels of metal accumulated represent optimum accumulation rates for healthy study species.

Phytoremediation Feasibility

The aim of the research project was to determine whether coppice woodland could offer an alternative option for the remediation of heavy metal contaminated soils. In this regard a theoretical assessment is discussed with regard to phytoremediation time scales.

The theoretical site is a one hectare site contaminated with zinc (1000 mg kg^{-1}) to a depth of one metre. In accordance with current guidelines (ICRCL, 1987) the phytoremediation of the site would require the reduction of the zinc concentration in the soil to 300 mg kg^{-1} in accordance with the ICRCL guidelines. How long would remediation take using the optimum accumulation results obtained from the three studies ?

Assume the volume of soil requiring treatment is 10000m^3 or approximately 20000 tonnes, therefore the trees have to remove 700 g per ton of soil which equates to 14000 kg of zinc per hectare). The maximum yield obtainable from coppice woodland has been assumed as 30t ha^{-1} y^{-1} (dry weight). The optimum remediation times are given in the following table:

Experimental Work	Maximum Zn conc in biomass (mg/kg)	Weight of Zn removed in 30t (kg)	Remediation time (years)
Field	588	17.64	794
Pot	2295	68.85	204
Hydroponics	5250	157.5	88

The results given in the table represent the shortest possible remediation times given the maximum concentration of zinc accumulated in the biomass samples recovered from each experimental study.

This short exercise indicates that the remediation of zinc contaminated soils could take between 88 and 794 years. However, it should also be noted that the results are optimum

remediation times and a conservative estimate of such remediation may be more than double the calculated times, as the documented yield is unlikely be obtained in contaminated soil and uptake values are maximum, not average. Therefore coppice woodland does not appear to offer a quick and cheap method to clean up contaminated land. It is worth noting that remediation times would be shorter if the level of reduction in contamination was smaller and in this regard phytoremediation may be more suited for the cleaning up of marginally contaminated land.

Additional Benefits and Applications

Coppice woodland offers a number of alternative benefits which have been highlighted. Such benefits include habitat creation, reduction of soil erosion, screening of unsightly sites, the provision of a physical barrier which can deter people from entering the site and the production of a marketable cash 'crop' generating a financial return from land which at present has no economic value. Subsequently when land prices increase or a developer wishes to redevelop a site the trees can be cleared quickly.

From the research undertaken the uptake of heavy metals by a number of study trees and seedlings was generally low. Such trees that exclude heavy metals are unlikely to help remediate contaminated soil but their selection may be beneficial in that any harvested wood would be uncontaminated and therefore there would be no restrictions on the use or disposal of the wood. Such trees would also pose less of a health risk to animals and insects which feed on tree leaves and sap.

Further Studies

The initial literature review carried out at the onset of this research project did not find relevant or directly applicable information on the phytoremediation of heavy metal contaminated land. Phytoremediation was a developing technology and a number of

researchers and institutions were undertaking various studies In the time since the commencement of the study a number of new publications have been issued. This short discussion will summarise some relevant publications arising since the completion of the practical work.

It is understood that plant breeding progammes have created many different willow and poplar clones. Punshon et al. (1995) screened sixteen *Salix* clones for copper resistance in an attempt to devise a selection method for willows capable of phytoremediation. At the time of writing it was stated that the UK National Willow Collection, Ness Botanic Gardens, South Wirral held upwards of 500 different willow clones. It has also been reported that the genetic improvement of tree species is likely to result in higher yielding species capable of accumulating higher concentrations of metal (Stomp et al., 1993).

Greger & Landberg (1999) discuss the use of willow in phytoextraction. Up to 150 clones of willow are believed to have been screened for uptake, transport and tolerance to Cd, Cu and Zn. The uptake of cadmium, copper and zinc varied between 0.2-8.5, 0.4-9.0 and 14-1813 mg kg^{-1} in shoots and 4.1-291, 14-331 and 72-2140 mg kg^{-1} in roots respectively.

The research in to phytoremediation feasibility has progressed from laboratory studies in to field scale studies. The uptake levels recorded by coppice woodland species appear relatively small when compared with levels in the soil. Felix (1997) discusses the *in situ* decontamination of heavy metal polluted soil (6.6 ppm Cd and 810 ppm Zn) with metal accumulating plants (*Thlaspi caerulescens, Alyssum murale* and *Salix viminalis*). From literature data and preliminary experiments it was concluded that yield and metal uptake rates of such plants would have to be increased to allow remediation within reasonable timescales. Such conclusions are similar to the findings discussed.

It has been suggested that coppice woodland management would allow derelict and contaminated sites to be cropped to give some economic return until a developer purchases a site. Other associated benefits include the creation of wildlife habitat, screening of unsightly sites and the provision of a barrier to would be trespassers.

Research by Hasselgren (1998) and Labrecque et al. (1998) report that coppice woodland can also be used for the disposal of pre-treated waste water, sewage sludge and methanogenic landfill leachate. These types of disposal options are important to local authorities and water authorities facing increasing restrictions on the disposal of such wastes. Coppice woodland may offer an alternative disposal route which also has a beneficial effect of yield and growth of the trees.

As previously discussed, while the 'complete' phytoremediation of a site by phytoremediation is likely to take many years, the studies within this thesis indicate that such cropping regimes are likely to remove the most available fractions of metals from the soil within a few years. In today's society more and more emphasis is placed on the assessment of risk when developing a site and determining appropriate clean up levels. Mobile and leachable contaminants are considered to present more of a risk to the environment when compared with metals tightly held in the soil. In this regard phytoremediation practices are likely to remove the 'worst' contamination. Eriksson & Ledin (1999) studied the effects of *Salix* cropping on the phytoavailability and concentration of cadmium in soil. The concentration of exchangeable cadmium throughout the soil profile (0-0.65m) was significantly lower in *Salix* stands than reference areas while total soil concentrations were only slightly depleted. The indicates that coppice cropping can reduce the levels of potentially leachable metals.

At the completion of the experimental works extensive field trials were being undertaken in Sweden to test and select high yield poplar and willow hybrids. Although these hybrids were not available during this study they offer attractive opportunities in the field of phytoremediation due to significant increases in yield. Speculative genetic engineering works were also being discussed and researchers were confident of transferring metal accumulating genes to other species. Such developments are likely to make phytoremediation a realistic option for the clean up of heavy metal contaminated sites in the future.

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APPENDIX 1 Tables 3.6 to 3.17. Results of 'Field Experiment'

Table 3.6. Moisture Content of Biomass Samples (Summerford)

Date	Birch (Tree 1)								Birch (Tree 2)								Birch (Tree 3)							
	4 ft				14 ft				4 ft				14 ft				4 ft				14 ft			
	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf
29 Mar	49.7	43.2	43.5		52.5	38.2	42.4		47.7	43.5	44.8		50.3	44.5	46.2		46	42.1	43.5		50.2	47.7	49.3	
29 Apr.	52.8	47.6	48.3	72.4	55.3	49.2	63.1	73.6	49.9	45.1	47.6	72.3	54.8	45.9	56.5	71.3	51.5	49.2	44.7	68.7	50.2	44.4	50.1	64.1
29 Jun.	49.2	36.6	44.9	62.0	55	44.7	52.4	64.2	44.1	35.6	44.5	60.2	57.8	48.8	55.9	62.8	50.6	44	45.3	61.4	58.1	51.6	56.6	63.2
29 Jul.	51.6	45.7	49.1	61.2	62.5	56.1	59.2	63.5	47.5	40.9	43.9	60.7	56.6	48.3	52.9	61.2	47.6	43.7	44.4	62.2	59.2	54.4	55.1	61.1
29 Aug.	48.5	44.9	43.8	61.3	54.8	48.7	50.4	61.2	44.8	43.4	41	59.5	51	46.6	45.9	57.5	46.9	44	40	58.8	55.1	52.6	51.2	58.9
29 Sept.	47.7	38.4	38.3	60.6	52.2	30.6	26.4	61.7	43.2	17.4	14.5	60	46.4	27.6	20.9	60.1	40.4	24	12.4	60.2	47.8	43.1	40.7	59.5
18 Oct.	47.3	43.5	41.6	61.5	51.1	46.1	43.2	62.1	42.4	42.5	37.8	68.3	45.1	44	42.2	62.1	42.2	40.5	37.5	60.6	43.6	53.6	40.7	64.1

Note: All values are expressed as percentages moisture contents (fresh weight)

Table 3.7. Moisture Content of Foliage Samples (Redding)

Date	Birch (front)				Birch (back)				Willow (front)				Willow (back)				Sycamore			
	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf
29 Mar	48.2	40.7	40.3		45.9	51.2	47.5		27.8				56.3	46.2	52.5		49.2	50.2	52.7	
29 Apr.	46.5	44.6	55.2	71.5	46.9	50.6	58.3	78.2	58.8	47	55.9	79.4	59.6	48.3	58	76	53.8	47.8	49.4	74.7
29 Jun.	46.9	42.2	44.7	62.4	52.2	48.2	48.4	66.2	58.4	47.6	56.4	64.8	61.1	48.8	57.2	63.1	55.1	43.9	48.5	70.7
29 Jul.	46.2	43.5	42.2	66.3	53	48.4	48.5	66.6	55.4	47.1	51.8	65.7	58.6	49.4	53.1	64.3	51.6	45.2	49.6	68.7
29 Aug.	48.6	45.5	45.1	61.9	52.6	51.9	47.8	63.1	55.7	48.7	52.7	64.9	55.9	48.5	52		51.5	46.6	50.6	68.2
29 Sept.	43.1	29.1	32.2	61.3	43.5	44.8	33.7	60.4	51.9	42.7	49.1	63.5	53	33.6	36.8	62.7	50.4	42.7	44.6	68.5
18 Oct.	42	43.1	40.5	59.1					51.6	45.3	49.1	67.7	52.4	50.2	47	62.4	50.6	45.1	47.4	66.9

Note: All values are expressed as percentage moisture contents(fresh weight)

Table 3.8. Zinc in Biomass Samples (Summerford)

Date	Birch (Tree 1)								Birch (Tree 2)								Birch (Tree 3)							
	4 ft				14 ft				4 ft				14 ft				4 ft				14 ft			
	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf
Mar-94	92	33	91		93	34	94		134	50	166		117	59	161		117	50	141		125	60	152	
Apr.-94	87	19	94	87	87	18	118	86	107	45	179	79	82	19	109	67	122	35	145	63	110	29	130	69
Jun.-94	69	21	68	130	80	24	100	113	97	37	152	132	89	22	132	95	99	34	137	137	116	26	152	87
Jul.-94	71	17	72	140	89	18	97	113	90	27	132	129	98	18	142	89	90	30	118	163	106	29	130	111
Aug.-94	74	28	79	166	90	25	104	136	110	48	107	154	96	33	113	72	96	41	117	148	101	42	129	104
Sep-94	108	27	81	217	98	26	83	131	109	46	137	165	114	43	139	72	124	53	125	166	114	48	125	114
Oct.-94	92	26	79	185	90	32	89	176	118	50	126	169	121	54	138	98	137	59	124	205	114	50	119	163
Apr.-95	56			136	109			127	124			74	148			127	118			87	135			78
May-95	89			211	98			167	134			154	133			144	132			193	142			171
Jun.-95	73			185	78			158	103			164	100			123	100			194	104			152
Aug-95	80			197	89			138	111			165	118			104	108			205	115			173
Sep-95	86			202	81			145	111			165	117			91	102			195	114			164
Oct.-95	106			242	108			216	113			207	130			123	119			245	123			197
Nov-95	95			294	110			239	112			248	133			122	112				121			215
Dec-95	102	37	87		101	43	143		114	48	115		128	57	149		107	39	106		121	71	170	
Jan-96	96	34	86		109	37	127		116	41	123		125	47	162		107	42	114		138	55	138	
Feb.-96	97	33	90		109	36	90		110	45	116		138	54	149		120	46	106		135	54	131	
Mar-96	111	30	100		103	40	103		120	30	100		133	57	177		104	47	120		155	67	155	

Note: All values are zinc concentrations expressed as mg kg^{-1} of oven dry plant biomass.

Table 3.9. Copper in Biomass Samples(Summerford)

Date	Birch (Tree 1)								Birch (Tree 2)								Birch (Tree 3)							
	4 ft				14 ft				4 ft				14 ft				4 ft				14 ft			
	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf
Mar-94	10	<5	8		7	5	7		11	<5	7		6	<5	8		5	<5	8		7	<5	8	
Apr.-94	<5	<5	6	10	6	<5	6	9	6	<5	5	12	5	<5	5	10	<5	<5	5	7	5	<5	5	10
Jun.-94	<5	<5	6	<5	<5	<5	5	4	<5	<5	<5	<5	5	<5	5	<5	<5	<5	<5	<5	<5	<5	5	<5
Jul.-94	7	<5	8	5	6	<5	6	6	6	<5	7	<5	6	<5	7	6	6	<5	6	5	6	<5	7	6
Aug-94	6	<5	7	<5	5	<5	<5	<5	5	<5	<5	<5	<5	<5	5	<5	5	<5	<5	<5	<5	<5	6	<5
Sep-94	9	<5	8	6	7	<5	8	8	6	<5	6	5	5	<5	<5	<5	6	<5	5	<5	5	5	6	5
Oct-94	14	7	11	7	10	6	10	8	11	5	9	8	9	6	9	7	8	<5	7	6	8	5	7	8
Apr.-95	10			16	6			11	5			8	60			11	<5			6	<5			<5
May-95	8			6	7			7	5			5	7			6	6			5	5			6
Jun.-95	5			6	6			<5	7			6	28			7	5			5	5			6
Aug-95	8			<5	7			7	6			5	6			5	7			<5	5			<5
Sep-95	8			<5	6			7	8			<5	7			5	6			<5	6			<5
Oct-95	8			<5	7			5	5			<5	6			<5	6			<5	5			<5
Nov-95	8			<5	6			<5	6			<5	5			<5	7				5			<5
Dec-95	8	<5	8		6	23	6		<5	<5	9		5	<5	5		<5	<5	5		6	<5	<5	
Jan-96	9	5	9		7	<5	10		6	<5	6		7	<5	6		5	<5	6		6	<5	6	
Feb.-96	9	<5	8		8	<5	10		9	<5	<5		8	6	6		6	<5	<5		7	<5	5	
Mar-96	9	<5	9		7	<5	6		6	<5	9		7	<5	5		6	5	5		6	5	6	

Note: All copper concentrations are expressed as mg kg^{-1} oven dry plant biomass.
Shaded values indicate likely errors

Table 3.10. Chromium in Biomass Samples (Summerford)

Date	Birch (Tree 1)								Birch (Tree 2)								Birch (Tree 3)							
	4 ft				14 ft				4 ft				14 ft				4 ft				14 ft			
	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf
Mar-94	<5	<5	<5	<5	<5	6	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5	
Apr-94	<5	<5	<5		<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Jun-94	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Jul-94	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Aug-94	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Sep-94	<5	<5	<5	<5	<5	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Oct-94	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Apr-95	<5			<5	<5	<5		<5	<5			<5	<5			<5	<5	<5	<5	<5	<5	<5	<5	<5
May-95	<5			<5	<5	<5		<5	<5			<5	<5			<5	<5			<5	<5			<5
Jun-95	<5			<5	<5	<5		<5	<5			<5	<5			<5	<5			<5	<5			<5
Aug-95	<5			<5	<5	<5		<5	<5			<5	<5			<5	<5			<5	<5			<5
Sep-95	<5			<5	<5	<5		<5	<5			<5	<5			<5	<5			<5	<5			<5
Oct-95	<5			<5	<5	<5		<5	<5			<5	<5			<5	<5			<5	<5			<5
Nov-95	<5			<5	<5	<5		<5	<5			<5	<5			<5	<5			<5	<5			<5
Dec-95	<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5	
Jan-96	<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5	
Feb-96	<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5	
Mar-96	<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5	

Note: All chromium concentrations are expressed as mg kg^{-1} of oven dry plant biomass

Table 3.11. Lead in Biomass Samples (Summerford)

Date	Birch (Tree 1)								Birch (Tree 2)								Birch (Tree 3)							
	4 ft				14 ft				4 ft				14 ft				4 ft				14 ft			
	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf	twig	wood	bark	leaf
Mar-94	<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20	
Apr-94	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Jun-94	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Jul-94	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Aug-94	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Sep-94	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Oct-94	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Apr-95	<20			<20	<20			<20	<20			<20	<20			<20	<20			<20	<20	<20	<20	<20
May-95	<20			<20	<20			<20	<20			<20	<20			<20	<20			<20	<20			<20
Jun-95	<20			<20	<20			<20	<20			<20	<20			<20	<20			<20	<20			<20
Aug-95	<20			<20	<20			<20	<20			<20	<20			<20	<20			<20	<20			<20
Sep-95	<20			<20	<20			<20	<20			<20	<20			<20	<20			<20	<20			<20
Oct-95	<20			<20	<20			<20	<20			<20	<20			<20	<20			<20	<20			<20
Nov-95	<20			<20	<20			<20	<20			<20	<20			<20	<20			<20	<20			<20
Dec-95	<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20	
Jan-96	<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20	
Feb-96	<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20	
Mar-96	<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20	

Note: All lead concentrations are in mg kg^{-1} oven dry biomass

Table 3.12. Zinc in Biomass Samples (Redding)

Date	Birch (Front)				Birch (Back)				Willow (Front)				Willow (Back)				Sycamore			
	Twig	Wood	Bark	Leaf	Twig	Wood	Bark	Leaf	Twig	Wood	Bark	Leaf	Twig	Wood	Bark	Leaf	Twig	Wood	Bark	Leaf
Mar-94	244	81	216		210	106	265		103	52	191		230	68	344		16	12	21	
Apr-94	204	40	218	100	194	95	229	114	140	15	178	89	260	33	314	108	24	22	17	57
Jun-94	148	36	202	166	193	44	275	335	94	23	155	74	177	23	243	156	19	8	20	27
Jul-94	169	41	197	183	189	46	266	588	102	37	154	73	172	38	227	163	15	4	18	32
Aug-94	190	54	187	172	212	80	221	461	118	36	144	80	217	38	252	212	13	6	18	21
Sep-94	207	56	199	220	213	88	255	580	103	28	158	96	169	36	228	187	14	5	17	25
Oct-94	205	48	189	269					95	25	115	82	153	34	204	183	17	4	19	28
Apr-95	227			56	216			83	147			59	192			103	6			30
May-95	217			193	237			337	167			131	257			245	16			31
Jun-95	190			188	234			241	120			121	169			136	8			27
Aug-95	193			196	194			359	124			117	163			157	15			26
Sep-95	198			169	165			402	105			116	165			218	12			23
Oct-95	180			196	210			450	117			133	159			183	17			25
Nov-95	220			235	193			503	124			123	143			158	28			24
Dec-95	200	62	209		182	58	205		139	25	173		217	71	302		17	8	21	
Jan-96	210	49	187		222	61	213		136	25	175		141	43	210		23	15	36	
Feb-96	196	59	174		218	65	187		160	42	221		189	59	276		17	8	22	
Mar-96	189	47	176		230	67	227		163	37	212		195	42	272		24	8	22	

Note: All zinc concentrations are expressed as mg kg^{-1} of oven dry biomass

Table 3.13. Copper in Biomass Samples (Redding)

Date	Birch (Front)				Birch (Back)				Willow (Front)				Willow (Back)				Sycamore			
	Twig	Wood	Bark	Leaf	Twig	Wood	Bark	Leaf	Twig	Wood	Bark	Leaf	Twig	Wood	Bark	Leaf	Twig	Wood	Bark	Leaf
Mar-94	8	<5	7		8	<5	6		14	<5	9		14	11	6		5	<5	6	
Apr-94	<5	<5	5	8	5	<5	<5	10	10	9	<5	16	13	13	<5	11	<5	<5	<5	6
Jun-94	<5	<5	5	<5	5	<5	<5	5	11	9	<5	7	10	8	<5	7	<5	<5	7	<5
Jul-94	7	<5	7	5	7	<5	9	7	12	15	<5	7	13	13	<5	9	5	<5	5	5
Aug-94	<5	<5	5	<5	6	<5	6	6	10	9	<5	5	12	10	<5	8	<5	<5	<5	<5
Sep-94	6	<5	5	<5	6	<5	5	8	12	6	5	6	12	10	6	6	<5	<5	<5	<5
Oct-94	7	<5	7	<5					12	12	6	6	14	14	6	7	<5	<5	<5	7
Apr-95	6			13	10			15	12			19	51			10	<5			9
May-95	7			5	10			7	12			13	12			14	<5			6
Jun-95	6			5	6			6	10			11	12			12	<5			6
Aug-95	7			<5	11			6	12			7	12			9	<5			<5
Sep-95	7			5	11			<5	13			8	11			9	5			<5
Oct-95	5			<5	8			<5	12			6	11			7	<5			<5
Nov-95	6			<5	10			6	10			7	10			7	<5			<5
Dec-95	<5	<5	5		12	<5	8		13	7	<5		8	14	7		<5	<5	<5	
Jan-96	6	<5	5		9	<5	8		14	8	5		12	12	5		6	<5	6	
Feb-96	6	<5	6		10	6	26		16	9	5		11	10	<5		<5	<5	<5	
Mar-96	5	<5	5		8	<5	9		14	9	<5		15	16	<5		5	<5	5	

Note: All copper concentrations are expressed as mg kg^{-1} oven dry weight

Table 3.14. Chromium in Biomass Samples(Redding)

Date	Birch (Front)				Birch (Back)				Willow (Front)				Willow (Back)				Sycamore			
	Twig	Wood	Bark	Leaf	Twig	Wood	Bark	Leaf	Twig	Wood	Bark	Leaf	Twig	Wood	Bark	Leaf	Twig	Wood	Bark	Leaf
Mar-94	<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5	
Apr-94	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Jun-94	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Jul-94	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Aug-94	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Sep-94	<5	<5	<5	<5	<5	7	<5	<5	<5	<5	<5	<5	<5	<5	<5	5	<5	<5	<5	<5
Oct-94	<5	<5	<5	<5					<5	<5	<5	<5	<5	7	<5	<5	<5	<5	<5	<5
Apr-95	<5			<5	<5			<5	<5			<5	<5	<5	<5	<5	<5	<5	<5	<5
May-95	<5			<5	<5			<5	<5			<5	<5			<5	<5			<5
Jun-95	<5			<5	<5			<5	<5			<5	<5			<5	<5			<5
Aug-95	<5			<5	<5			<5	<5			<5	<5			<5	<5			<5
Sep-95	<5			<5	<5			<5	<5			<5	<5			<5	<5			<5
Oct-95	<5			<5	<5			<5	<5			<5	<5			<5	<5			<5
Nov-95	<5			<5	<5			<5	<5			<5	<5			<5	<5			<5
Dec-95	<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5	
Jan-96	<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5	
Feb-96	<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5	
Mar-96	<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5		<5	<5	<5	

Note: All chromium concentrations are expressed as mg kg^{-1} of biomass dry weight

Table 3.15. Lead in Biomass Samples (Redding)

	Birch (Front)				Birch (Back)				Willow (Front)				Willow (Back)				Sycamore			
	Twig	Wood	Bark	Leaf	Twig	Wood	Bark	Leaf	Twig	Wood	Bark	Leaf	Twig	Wood	Bark	Leaf	Twig	Wood	Bark	Leaf
Mar-94	37	26	41		41	25	62		32	<20	23		<20	<20	<20		25	<20	<20	
Apr-94	25	<20	25	<20	38	<20	56	<20	<20	<20	<20	<20	22	<20	22	<20	<20	<20	20	<20
Jun-94	21	<20	<20	<20	24	<20	36	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Jul-94	21	<20	24	<20	21	<20	26	<20	<20	<20	<20	<20	<20	<20	<20	<20	29	<20	26	<20
Aug-94	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Sep-94	27	<20	30	<20	40	<20	42	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Oct-94	23	<20	<20	<20					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Apr-95	31			<20	<20			<20	<20			<20	20			<20	<20			<20
May-95	21			<20	<20			<20	<20			<20	<20			<20	<20			<20
Jun-95	<20			<20	<20			<20	<20			<20	<20			<20	<20			<20
Aug-95	33			<20	<20			<20	<20			<20	<20			<20	<20			<20
Sep-95	27			<20	<20			<20	<20			<20	<20			<20	<20			<20
Oct-95	28			<20	<20			<20	<20			<20	<20			<20	<20			<20
Nov-95	25			<20	<20			<20	<20			<20	<20			<20	<20			<20
Dec-95	22	<20	21		<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20	
Jan-96	23	<20	28		<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	21	
Feb-96	<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	20	
Mar-96	<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20		<20	<20	<20	

Note: All lead concentrations are expressed as mg kg^{-1} biomass oven dry weight

Table 3.16. Zinc, Copper, Lead and Chromium in Root Samples (Summerford and Redding)

Date	Birch (tree 1)		Birch (Tree 2)		Birch (Tree 3)		Birch (Front)		Birch (Back)		Willow (Front)		Willow (Back)		Sycamore	
	fine root	large root	fine root	large root	fine root	large root	fine root	large root	fine root	large root	fine root	large root	fine root	large root	fine root	large root
								Zinc								
Mar-94	41	45	29	48	51	101	147	116	152	135	134	122	141	161	345	44
April-94	30		46		58	22	152		139		90		123		90	
Jun-94	14	13	21	29		24	111	79	86	151	128	99	185	99	48	20
Jul-94	28	16	26	29	37	44	129	138	132	125	117	119	164	107	52	33
Aug-94	44	40	46	33	38	47	118	94	66	88	88	88	128	119	80	35
Sept-94	52	39	28	38	46	38	100	66	113	147	123	76	185	113	107	33
Oct-94	30	25	44	51	52	51	117	101	132	124	82	81	141	110	916	141
								Copper								
Mar-94	22	<5	31	7	12	7	15	11	12	6	80	12	38	12	61	13
April-94	5		16		<5	10	9		8		5		41		16	
Jun-94	<5	<5	7	5		<5	8	5	9	104	11	6	32	8	19	5
Jul-94	6	<5	7	<5	7	6	14	8	14	8	20	12	31	25	12	9
Aug-94	26	<5	8	5	6	<5	7	<5	16	8	11	7	8	<5	21	10
Sept-94	7.9	5	10	<5	10	<5	9	5	10	<5	12	8	14	8	32	12
Oct-94	22	9	11	8	8	6	20	7	8	6	12	8	34	13	26	7
								Lead								
Mar-94	51	<20	<20	<20	30	<20	132	31	95	35	265	62	100	16	2920	219
April-94	<20		30		<20	85	146		74		82		81		402	
Jun-94	<20	<20	<20	20		39	307	<20	39	21	158	213	54	312	255	21
Jul-94	<20	<20	<20	<20	<20	<20	98	58	107	53	573	199	128	92	84	37
Aug-94	29	<20	<20	<20	<20	<20	190	68	83	48	152	70	79	76	292	224
Sept-94	<20	<20	<20	<20	24	<20	116	34	355	767	175	41	124	56	436	133
Oct-94	31	<20	<20	<20	<20	<20	102	38	167	86	119	76	94	22	615	215
								Chromium								
Mar-94	157	9	145	25	66	15	13	<5	14	<5	10	<5	88	5	<5	<5
April-94	19		151		11	425	<5		<5		<5		<5		<5	
Jun-94	18	8	32	21		25	<5	<5	<5	<5	<5	<5	5	<5	<5	<5
Jul-94	20	9	50	8	13	9	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Aug-94	67	10	57	16	34	14	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Sept-94	23	13	92	14	61	19	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Oct-94	95	9	30	18	23	11	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5

Note: All metal concentration are expressed as mg kg^{-1} oven dry weight

Table 3.17. Zinc, Copper, Chromium and Lead in Biomass Samples (Site H4, Redding)

	Redding H4 (Birch)						Redding H4 (Willow)					
	fine Root	large root	twig	wood	bark	leaf	fine Root	large root	twig	wood	bark	leaf
						Zinc						
Mar-94	293	171	231	66	274		327	125	161	67	333	
Apr-94												
Jun-94			216	39	261	219			171	41	324	159
Jul-94	108	78	202	33	240	278	130	80	173	44	259	170
Aug-94	1120	469	244	59	260	358	180	69	164	43	250	190
Sep-94	141	87	242	79	281	316	75	44	182	42	273	196
Oct-94	76	64	229	53	229	425	196	124	159	42	247	223
						Copper						
Mar-94	2610	358	10	<5	7		1190	249	15	<5	12	
Apr-94												
Jun-94			11	<5	9	5			13	11	<5	9
Jul-94	294	32	10	6	10	7	497	131	16	21	6	9
Aug-94	7290	1700	10	<5	7	15	875	91.2	13	12	6	8
Sep-94	842	100	11	<5	11	5	445	85.8	15	14	8	7
Oct-94	120	42	11	5	10	6	909	249	15	17	8	7
						Chromium						
Mar-94	8.0	<5	,5	6	<5		<5	<5	<5	6	<5	
Apr-94												
Jun-94			<5	<5	<5	<5			<5	<5	<5	<5
Jul-94	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Aug-94	<5	<5	<5	<5	<5	19	<5	<5	<5	<5	<5	12
Sep-94	<5	<5	<5	5	<5	<5	<5	<5	<5	<5	<5	<5
Oct-94	6	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
						Lead						
Mar-94	1690	208	<20	29	<20		937	232	<20	29	<20	
Apr-94												
Jun-94			20	<20	25	<20			<20	<20	<20	<20
Jul-94	159	32	<20	<20	<20	<20	391	114	<20	<20	<20	<20
Aug-94	5050	725	<20	<20	<20	<20	170	<20	<20	<20	<20	<20
Sep-94	527	108	<20	<20	22	<20	54	<20	<20	<20	<20	<20
Oct-94	37	<20	<20	<20	<20	<20	597	207	<20	<20	<20	<20

Note: All metal concentrations are expressed as mg kg^{-1} oven dry weight

APPENDIX 2 Tables 4.10 to 4.14. 'Pot Experiment'

Table 4.10. The Concentration Of Metal Extracted By 0.01M Calcium Chloride

Soil Metal Treatments	*Salix viminalis*		*Pinus contorta*		*Alnus incana*		*Populus trichocarpa*	
	1995	1996	1995	1996	1995	1996	1995	1996
500 zinc	566 ± 19	327 ± 20	598 ± 10	340 ± 1	494 ± 5	399 ± 6	500 ± 23	349 ± 13
3000 zinc	3192 ± 167	1805 ± 68	3874 ± 130	4485 ± 184	3705 ± 133	2768 ± 210	3142 ± 41	2641 ± 20
500 copper	25 ± 0	36 ± 1	40 ± 1	26 ± 2	46 ± 2	31 ± 0	33 ± 1	29 ± 0
2000 copper	373 ± 8	337 ± 1	600 ± 18	274 ± 10	450 ± 2	368 ± 10	429 ± 14	251 ± 12
500 nickel	736 ± 27	315 ± 2	791 ±24	351 ± 6	666 ± 3	644 ± 13	740 ± 14	341 ± 1
1000 nickel	1430 ± 24	1224 ± 0	1673 ± 19	843 ± 21	1648 ± 34	1055 ± 22	1966 ± 14	779 ± 61
300 cadmium	339 ± 4	222 ± 1	306 ± 10	225 ± 8	324 ± 13	287 ± 8	363 ± 1	198 ± 1
1000 cadmium	1655 ± 21	581 ± 80	1480 ± 27	1141 ± 36	1423 ± 27	689 ± 26	1821 ± 23	667 ± 56
2000 lead	138 ± 1	89 ± 6	171 ± 0	154 ± 1	228 ±23	162 ± 4	101 ± 2	134 ± 5
2000 chromium	513 ± 9	1223 ± 65	802 ± 11	276 ± 15	1397 ±41	54 ± 0	760 ± 11	159 ± 4

NOTE: All values represent soil metal concentrations (mg kg^{-1})

Table 4.11. The Concentration Of Metal Extracted By 0.05M Ammonium EDTA

Soil Metal Treatments	*Salix viminalis*		*Pinus contorta*		*Alnus incana*		*Populus trichocarpa*	
	1995	1996	1995	1996	1995	1996	1995	1996
500 zinc	802 ± 3	422 ± 40	697 ± 2	481 ± 14	692 ± 10	589 ±23	705 ± 2	507 ± 9
3000 zinc	4475 ± 215	2426 ± 104	3987 ± 69	5296 ± 281	4326 ± 226	4015 ±192	3672 ± 143	3468 ± 24
500 copper	525 ± 192	527 ± 18	520 ± 5	573 ± 26	543 ± 43	405 ± 18	428 ± 4	534 ± 10
2000 copper	1783 ± 5	1768 ± 20	2283 ± 90	1693 ± 50	1924 ± 45	1634 ± 19	1991 ± 19	1846 ± 1
500 nickel	927 ± 35	538 ± 107	984 ± 19	498 ± 10	832 ± 12	781 ± 33	887 ± 24	485 ± 21
1000 nickel	1724 ± 90	1565 ± 111	1986 ± 18	1065 ± 35	1926 ± 19	1317 ± 1	2253 ± 97	931 ± 15
300 cadmium	445 ± 35	338 ± 9	405 ± 9	319 ± 12	445 ± 17	400 ± 9	431 ± 19	323 ± 3
1000 cadmium	1885 ± 19	880 ± 15	1862 ± 43	1574 ± 12	1816 ± 22	1031 ± 18	2036 ± 75	987 ± 7
2000 lead	2749 ± 104	2325 ± 29	3079 ± 123	2358 ± 1	3823 ± 137	2925 ± 190	2781 ± 32	3123 ± 34
2000 chromium	814 ± 61	293 ± 17	1225 ± 30	554 ± 7	2299 ± 498	181 ± 0	1189 ± 6	356 ± 5

NOTE: All values represent soil metal concentrations (mg kg^{-1})

Table 4.12. Residual Metal Determined By Acid Digestion

Soil Metal Treatments	*Salix viminalis*		*Pinus contorta*		*Alnus incana*		*Populus trichocarpa*	
	1995	1996	1995	1996	1995	1996	1995	1996
500 zinc	935 ± 52	713 ± 30	888 ± 1	610 ± 24	848 ± 5	955 ± 62	885 ± 34	651 ± 16
3000 zinc	4470 ± 35	2856 ± 71	4410 ± 364	5789 ± 435	4454 ± 155	4511 ± 815	3983 ± 35	3631 ± 46
500 copper	1194 ± 452	673 ± 17	844 ± 136	651 ± 38	737 ± 100	508 ± 7	628 ± 38	633 ± 25
2000 copper	2164 ± 30	2218 ± 76	2481 ± 8	1910 ± 5	2233 ± 151	2014 ± 84	2437 ± 54	1963 ± 104
500 nickel	928 ± 32	569 ± 28	1067 ± 12	580 ± 17	879 ± 35	905 ± 3	1058 ± 160	533 ± 8
1000 nickel	1731 ± 128	1455 ± 84	1920 ± 100	1272 ± 42	1972 ± 14	1555 ± 15	2277 ± 113	1092 ± 28
300 cadmium	473 ± 12	379 ± 40	444 ± 9	357 ± 3	483 ± 2	428 ± 8	489 ± 5	359 ± 5
1000 cadmium	2076 ± 3	1045 ± 8	1884 ± 20	1502 ± 5	1837 ± 39	1261 ± 25	2285 ± 23	1051 ± 8
2000 lead	3010 ± 26	3307 ± 82	3556 ± 527	3318 ± 336	4139 ± 113	4076 ± 32	3140 ± 185	4091 ± 140
2000 chromium	3128 ± 17	3237 ± 111	3851 ± 101	4120 ± 118	4811 ± 92	3538 ± 310	4007 ± 16	3466 ± 233

NOTE: All values represent soil metal concentrations (mg kg^{-1})

Table 4.13. The Metal Content In Foliage Samples (mg kg^{-1} Oven Dry Weight)

Soil Metal Treatment	*Salix viminalis*			*Pinus contorta*			*Alnus incana*			*Populus trichocarpa*		
	1994	1995	1996	1994	1995	1996	1994	1995	1996	1994	1995	1996
500 zinc	2995	1550	NS	215	459	82	NS	1350	412	1163	2330	NS
3000 zinc	NS	2455	NS	1236	886	NS	NS	368	NS	3756	NS	NS
500 copper	9	20	21	11	4.6	8	NS	11	18	8.3	7	15
2000 copper	NS	17	NS	72	34	NS	NS	30	NS	259	15	NS
500 nickel	NS	338	NS	63	28	NS	NS	779	NS	26	63	NS
1000 nickel	NS	630	NS	236	464	NS	NS	684	NS	36	NS	NS
300 cadmium	346	453	NS	34	161	273	NS	54	4	15	92	NS
1000 cadmium	NS	281	NS	229	272	NS	NS	97	NS	321	NS	NS
2000 lead	<20	<20	<20	<20	<20	<20	NS	<20	<20	<20	<20	31
2000 chromium	NS	126	NS	386	184	NS	NS	202	35	2274	20	NS

NOTE NS denotes treatments where no sample was collected

Table 4.14. The Metal Content Of Different Components Of Surviving Seedlings (August 1996)

Soil Metal Treatment	*Salix viminalis*			*Pinus contorta*			*Alnus incana*			*Populus trichocarpa*		
	wood	bark	leaves	wood	bark	leaves	wood	bark	leaves	wood	bark	leaves
500 zinc	NS	NS	NS	223	1227	82	571	1155	412	NS	NS	NS
500 copper	10	10	21	5	13	8	5	16	18	<5	16	15
300 cadmium	NS	NS	NS	172	384	273	58	384	4	NS	NS	NS
2000 lead	51	35	<20	<20	30	<20	29	26	<20	59	43	31
2000 chromium	NS	NS	NS	NS	NS	NS	12	263	35	NS	NS	NS

NOTE: NS denotes no samples collected for particular treatment

Values are expressed as mg kg^{-1} oven dry weight

APPENDIX 3 Tables 5.1 to 5.6. 'Hydroponic Experiment'

Table 5.1 Zinc in Sampled Biomass

Tree Species	Solution Metal Conc	Leaves/Needles			Final Harvest				
	($mg\ l^{-1}$)	Day 1	Day 4	Day 7	Leaves/ Needles	Twigs	Green Twigs	Old Twigs	Roots
Betula pendula	10	40 ± 1		34 ± 1	43 ± 1	130 ± 1			757 ± 120
	100	85		98 ± 5	146 ± 2	158 ± 10			2370 ± 117
	500	75		90 ± 2		639 ± 79			6890 ± 404
Pinus contorta	10	45 ± 5		44 ± 7	39 ± 0.3	40 ± 1			779 ± 26
	100	62 ± 2		61 ± 1	71 ± 2	138 ± 7			5950 ± 290
	500	75 ± 7		86 ± 1	70 ± 1	358 ± 2			12600 ±823
Alnus incana	10	20 ± 2		17	107 ± 1	132 ± 9			1220 ± 6
	100	18 ± 2		17 ± 2	437	262 ± 11			7820 ± 103
	500	27		32		192 ± 2			10400 ±316
Alnus rubra	10	9 ± 3		13 ± 6	30 ± 1	33 ± 1			1010 ± 176
	100	14		14		14 ± 2			8470 ± 480
	500	8 ± 1		10 ± 0.1		434 ± 23			20200 ±690
Alnus glutinosa	10	<1			1220	83 ± 5			950 ± 207
	100	50		50	1470	77 ± 3			4750 ± 340
	500			131		34 ± 2			11200 ±1770
Populus euroamericana	10	28 ± 13	48 ± 3	48 ± 18	215 ± 5		179 ± 4	195 ± 3	1100 ± 99
	100	28 ± 9	30 ± 0.1	50 ± 1	266 ± 1		187 ±5	426 ± 66	10100 ± 214
	500	23 ± 6	1030 ± 3	2360 ± 170			5250 ± 225	5010±445	15600 ±113
Populus trichocarpa	10	29 ± 4	39 ± 3	41 ± 6	119 ± 8		80 ± 3	94 ± 3	2420 ± 153
	100	26 ± 5	40 ± 3	101 ± 1	489 ± 3		436 ± 7	609 ± 28	8300 ± 23
	500	25 ± 1	2650 ± 200	1750 ± 130			3860 ± 45	3230 ± 8	12800 ±860
Salix caprea	10	17 ± 2	35 ± 2	116 ± 1	347 ± 20		265 ± 5	225 ± 11	1500 ± 3
	100	20 ± 0.1	21 ± 4	21 ± 0.1			44 ± 3	110 ± 8	7470 ± 125
	500	18 ± 1	56 ± 0.2	55 ± 12			174 ± 4	553 ± 137	12200 ± 8
Salix viminalis	10	17 ± 3	28 ± 2	29 ± 11	313 ± 8		123 ± 2	117 ± 12	2150 ± 60
	100	15 ± 0.3	19 ± 1	21 ± 1			38 ± 1	39 ± 1	7650 ± 47
	500	16 ± 4	63 ± 2	68 ± 5			217 ± 11	528 ± 237	14900 ± 2560

Table 5.2 Copper in Sampled Biomass

Tree Species	Solution Metal Conc	Leaves/Needles			Final Harvest				
	($mg\ l^{-1}$)	Day 1	Day 4	Day 7	Leaves/ Needles	Twigs	Green Twigs	Old Twigs	Roots
Betula pendula	10	<5		<5	<5 ± 0	5 ± 0.1			1690 ± 19
	100	<5		5	6 ± 2	<5 ± 0			6180 ±470
	500	<5 ± 0.1		15	49	582 ± 28			8150 ±1580
Pinus contorta	10	<5 ± 0		<5 ± 0	6 ± 0.7	8 ± 0.4			2210 ± 64
	100	<5 ± 0		<5 ± 0	8 ± 0.7	11 ± 0.2			11000 ±370
	500	<5 ± 0		75 ± 0.8	43 ± 4	335 ± 15			15700 ±1500
Alnus incana	10	8 ± 0		13 ± 3	8 ± 0.5	6 ± 0			1930 ± 184
	100	14 ± 0.1		14 ± 0.1	13	6 ± 0			5550 ± 1050
	500	9 ± 0.5		11		26 ± 2			14900 ±2300
Alnus rubra	10	<5 ± 0		<5 ± 0	<5 ± 0.4	6 ± 0.1			2420 ± 92
	100	<5 ± 0.1		<5 ± 0		<5 ± 0.8			8860 ± 384
	500	<5 ± 0		<5 ± 0.7		<5 ± 0			12400 ±1870
Alnus glutinosa	10	6				5 ± 0			1670 ± 512
	100	9		14		5 ± 0			8570 ± 1880
	500	<5 ± 0		8 ± 1.3	18	260 ± 25			14000 ± 680
Populus euroamericana	10	5 ± 0.2	7 ± 0.1	8 ± 0.8	8 ± 0.6		9 ± 0.7	9 ± 0.3	3250 ± 60
	100	8 ± 0.2	32 ± 1.8	37 ± 3.3	17.5 ± 0.1		154 ± 6	406 ± 67	9450 ± 419
	500	27 ± 0.1	128 ± 5.2	102 ± 2.3	34		407 ± 0.3	1340 ± 22	16500 ± 1500
Populus trichocarpa	10	6 ± 1.1	8 ± 0.1	9 ± 0.6	11 ± 0		6 ± 0.1	<5 ± 0.1	1210 ± 21
	100	13 ± 0.7	14 ± 1.1	26 ± 1.5	17 ± 1		80 ± 4	267 ± 11	9070 ± 94
	500	52 ± 1.2	299 ± 64	275 ± 17	32 ± 0.6		967 ± 17	959 ± 23	13100 ±367
Salix caprea	10	<5 ± 0	6 ± 0.6	5 ± 0	15 ± 0.2		5 ± 0.7	6 ± 0.8	2570 ± 100
	100	26 ± 0.1	75 ± 7.4	136 ± 15	43 ± 3		351 ± 22	342 ± 1	8010 ± 980
	500	291 ± 3	3140 ± 680	5080 ± 268	7140 ± 196		4530 ± 57	3740 ± 201	16400 ± 1550
Salix viminalis	10	6 ± 0	5 ± 0	5 ± 0	31 ± 0		<5 ± 0	<5 ± 0.1	3280 ± 23
	100	9 ± 0	11 ± 0.5	12 ± 0	32 ± 0.9		39 ± 0.5	58 ± 0.8	11500 ± 370
	500	183 ± 0.5	2760 ± 464	3720 ± 102	3580 ± 560		3440 ± 39	3840 ± 232	19600 ± 777

Table 5.3 Cadmium in Sampled Biomass

Tree Species	Solution Metal Conc	Leaves/Needles			Final Harvest				
	($mg\ l^{-1}$)	Day 1	Day 4	Day 7	Leaves/ Needles	Twigs	Green Twigs	Old Twigs	Roots
Betula pendula	10	<3		<3	<3	<3			883 ± 66
	100	<3		<3		<3			5960 ± 832
	500	<3		<3		40 ± 1.6			6600 ± 356
Pinus contorta	10	<3		<3	<3	<3 ± 1.4			1040 ± 12
	100	<3			<3	3 ± 0.1			11200 ± 518
	500	<3		41 ± 2.7	22 ± 3.6	951 ± 137			15800 ± 2930
Alnus incana	10	<3		<3		<3 ± 0			1850 ± 166
	100	<3		<3		3 ± 0.5			13900 ± 777
	500	<3		<3		4 ± 0.7			15600 ± 268
Alnus rubra	10	<3		<3		<3 ± 0			1990 ± 319
	100	<3		<3		<3 ± 0			13500 ± 1700
	500	<3		<3		<3 ± 0			17600 ± 364
Alnus glutinosa	10	<3		<3	<3	10 ± 7			1290 ± 216
	100	<3				<3			11300 ± 1500
	500	<3				<3			14700 ± 1720
Populus euroamericana	10	<3	<3 ± 0.7	<3	<3 ± 0		<3	4 ± 0	2390 ± 102
	100	<3	<3 ± 0.7	<3	5		<3	98 ± 0.5	16100 ± 109
	500	<3	71 ± 4	113 ± 21			508 ± 28	1590 ± 190	21400 ± 292
Populus trichocarpa	10	<3	<3 ± 0	<3	<3 ± 0.7		<3 ± 0.7	5 ± 0.1	1850 ± 137
	100	<3	<3	<3	10 ± 0.4		<3 ± 2.1	169 ± 8	14400 ± 381
	500	<3	3 ± 0	5 ± 0.7			5 ± 0	737 ± 16	16900 ± 183
Salix caprea	10	6 ± 0.7	<3	<3	11 ± 0		3 ± 0	9 ± 1	2270 ± 19
	100	<3	<3 ± 0.7	<3	<3		<3	<3 ± 0	11900 ± 648
	500	<3	<3	<3			<3 ± 0.7	24 ± 7	17600 ± 1010
Salix viminalis	10	<3	<3	<3	7 ± 3.7		8 ± 0	31 ± 0.7	2750 ± 329
	100	<3 ± 0	<3 ± 0	<3	<1		<3 ± 0.7	<3 ± 0	15000 ± 89
	500	<3	<3 ± 0	<3			<3 ± 0	147 ± 9	20300 ± 1470

Table 5.4 Nickel in Sampled Biomass

Tree Species	Solution Metal Conc	Leaves/Needles			Final Harvest				
	($mg\ l^{-1}$)	Day 1	Day 4	Day 7	Leaves/ Needles	Twigs	Green Twigs	Old Twigs	Roots
Betula pendula	10	<7		<7	<7 ± 0.3	<7 ± 2.7			615 ± 4.4
	100			<7	<7 ± 0	8 ± 0.2			2710 ± 148
	500	<7		<7	36 ± 0	118 ± 2.5			4420 ± 1020
Pinus contorta	10	<7		<7 ± 0.7	10 ± 0.6	42 ± 1.3			1330 ± 96
	100	<7 ± 1.5		11 ± 2.1	31 ± 3.5	87 ± 115			6740 ± 330
	500	<7		84 ± 24	259 ± 9	683 ± 51			8320 ± 683
Alnus incana	10	11 ± 1.9		<7 ± 0.7	7	<7 ± 0			2230 ± 124
	100	11 ± 0.1		<7	29 ± 0.6	<7 ± 0.7			7130 ± 303
	500	<7 ± 0		<7		23 ± 2.1			8681 ± 658
Alnus rubra	10	<7 ± 2		<7	<7	<7 ± 0.6			2300 ± 145
	100	<7 ± 2.4		<7	<7 ± 1.4	<7 ± 1.5			5010 ± 536
	500	<7		<7	<7 ± 2.7	16 ± 0.4			8620 ± 40
Alnus glutinosa	10	<7		40	<7	11 ± 0.7			1090 ± 9
	100	8		<7	<7	32 ± 0.5			4520 ± 20
	500	<7		22		134 ± 44			5360 ± 35
Populus euroamericana	10	<7 ± 3.5	14 ± 1.4	<7 ± 0	11 ± 0.7		13 ± 2.1	23 ± 0.4	2700 ± 14
	100	11 ± 1.3	28 ± 18	65 ± 12	72 ± 9		104 ± 7	399 ± 7	3460 ± 342
	500	14 ± 0.7	261 ± 7	859 ± 148	621 ± 18		812 ± 9	1010 ± 17	9450 ± 936
Populus trichocarpa	10	9 ± 2	<7 ± 2.1	10 ± 12	10 ± 2.7		<7 ± 0	17 ± 11	1210 ± 103
	100	11 ± 0.1	16 ± 0.6	51 ± 5	165 ± 12		161 ± 0.6	430 ± 12	6530 ± 260
	500	12 ± 5.6	790 ± 76	627 ± 7	604 ± 11		738 ± 11	889 ± 50	9900 ± 414
Salix caprea	10	8 ± 2.2	11 ± 4.3	22 ± 5	55		10 ± 1.8	46 ± 2.8	1760 ± 2.8
	100	<7 ± 0	<7 ± 1.4	<7 ± 3.5	14 ± 1.8		7 ± 1.5	68 ± 4.5	6350 ± 46
	500	<7 ± 1.4	633 ± 5	563 ± 16	1790 ± 38		365 ± 7	1090 ± 8	9290 ± 450
Salix viminalis	10	<7 ± 0	25 ± 8	44 ± 3.9	140 ± 0.1		40 ± 0.7	27 ± 1	2540 ± 93
	100	<7 ± 1.4	<7 ± 0.7	<7 ± 1.4	<7 ± 0.7		<7 ± 1.4	19 ± 14	8980 ± 129
	500	<7 ± 4.2	17 ± 2.9	16 ± 2.1	89 ± 7		42 ± 5	425 ± 18	10200 ± 1200

Table 5.5 Chromium in Sampled Biomass

Tree Species	Solution Metal Conc	Leaves/Needles			Final Harvest				
	($mg\ l^{-1}$)	Day 1	Day 4	Day 7	Leaves/ Needles	Twigs	Green Twigs	Old Twigs	Roots
Betula pendula	10	<5		<5	<5 ± 0	<5			610 ± 73
	100	<5		7	22 ± 0.4	<5 ± 0.7			2210 ± 340
	500	<5		163 ± 5	293	363 ± 33			9710 ± 811
Pinus contorta	10	<5 ± 0.7		<5 ± 0.7	7 ± 5	<5			3030 ± 327
	100	<5 ± 0.7		21 ± 0.6	128 ± 3	126 ± 2.4			10300 ± 76
	500	5 ± 0.7		891 ± 38	946 ± 30	2250 ± 386			30200 ± 2620
Alnus incana	10	<5		<5 ± 0.7	7 ± 0.4	<5			1720 ± 174
	100	<5		<5		<5			10800 ± 1070
	500	<5 ± 0.7		11 ± 0.6	77	<5 ± 1.4			24900 ± 1020
Alnus rubra	10	<5		<5 ± 0.7	10 ± 0.4	<5			1130 ± 135
	100	<5 ± 0.2		6 ± 0.2		<5 ± 0			8090 ± 204
	500	<5 ± 0.4		12 ± 0.8		19 ± 0.9			20600 ± 121
Alnus glutinosa	10	<5 ± 0.9		<5	13	<5 ± 0			1010 ± 34
	100	<5		18	23	<5 ± 0.7			6890 ± 543
	500	12		35		7 ± 0.1			13400 ± 830
Populus euroamericana	10	<5	<5	<5	<5 ± 0.1		<5	<5 ± 0.7	3600 ± 398
	100	11 ± 0.8	447 ± 6	663 ± 15	529 ± 27		579 ± 24	361 ± 6	18600 ± 969
	500	826 ± 39	4230 ± 112	3670 ± 30	2910 ± 28		1220 ± 89	2100 ± 36	31200 ± 749
Populus trichocarpa	10	<5 ± 0	<5	<5 ± 0.7	9 ± 0		<5	<5 ± 0.7	1570 ± 408
	100	<5 ± 0	180 ± 9	139 ± 6.4	467 ± 2.7		318 ± 40	266 ± 11	9810 ± 997
	500	23 ± 0.2	1110 ± 49	1260 ± 130	1460 ± 14		1200 ± 21	601 ± 86	27400 ± 1926
Salix caprea	10	<5	<5	<5	8 ± 0.7		<5	<5	2860 ± 104
	100	<5 ± 0.7	<5 ± 0	<5 ± 0	51 ± 2.4		<5 ± 0.7	<5 ± 0.6	11500 ± 214
	500	91 ± 9	3520 ± 144	3060 ± 39			1670 ± 122	1170 ± 23	30800 ± 146
Salix viminalis	10	<5	<5	<5	5 ± 0		<5	<5	3490 ± 107
	100	<5 ± 0	<5 ± 0.7	<5 ± 0	53 ± 1.1		<5 ± 0	22 ± 2.7	12200 ± 411
	500	107 ± 1.4	1290 ± 77	1530 ± 132	751		724 ± 14	1280 ± 120	33200 ± 4890

Table 5.6 Lead in Sampled Biomass

Tree Species	Solution Metal Conc	Leaves/Needles			Final Harvest				
	($mg\ l^{-1}$)	Day 1	Day 4	Day 7	Leaves/ Needles	Twigs	Green Twigs	Old Twigs	Roots
Betula pendula	10	<20 ± 11		<20	<20 ± 1.8	<20			1200 ± 252
	100	<20		<20 ± 2.1	<20	<20 ± 3.4			10900 ± 235
	500	<20 ± 0.7		<20 ± 0	<20 ± 4.1	<20 ± 1.4			34200 ± 3310
Pinus contorta	10	<20 ± 1.4		<20 ± 1.4	<20 ± 2	<20			1800 ± 127
	100	<20		<20 ± 3.5	<20	<20 ± 0			19500 ± 1090
	500	<20		<20 ± 2.9	<20	<20			42600 ± 785
Alnus incana	10	<20 ± 13		<20	<20 ± 0.2	<20			1960 ± 97
	100	<20		<20 ± 0.1	<20 ± 4.5	<20			17100 ± 22
	500	<20 ± 2.1		<20 ± 0.7	<20	<20			40800 ± 2760
Alnus rubra	10	<20 ± 2.8		<20	<20 ± 1.4	<20			2860 ± 155
	100	<20		<20 ± 2.5	<20	<20 ± 1.4			19900 ± 1820
	500	<20 ± 6		<20 ± 0.8		<20			37570±15000
Alnus glutinosa	10	<20		<20	29 ± 2.3	25 ± 1.1			1980 ± 151
	100	<20		<20	<20	<20			16500 ± 2550
	500	<20		<20 ± 9	<20	<20			30300 ± 3805
Populus euroamericana	10	<20	<20	<20	<20 ± 2.6		24 ± 0.9	35 ± 2.8	3650 ± 243
	100	<20 ± 1.5	<20 ± 1.5	<20 ± 5	<20 ± 3.5		<20	<20	37600 ± 494
	500	<20	<20 ± 2.1	<20	<20		60 ± 1.4	364 ± 0.3	68400 ± 842
Populus trichocarpa	10	<20 ± 0.7	<20	<20	<20 ± 0		<20 ± 0.4	<20 ± 1.5	1960 ±155
	100	<20 ± 2.9	<20 ± 1.5	<20 ± 9			<20 ± 2.7	22 ± 0.7	25900 ± 1200
	500	<20 ± 2.1	<20 ± 3.5	<20 ± 0.7	<20 ± 4.1		<20	<20	52000 ± 3270
Salix caprea	10	<20 ± 0	<20	<20 ± 2.1	<20 ± 1.4		<20 ± 0.6	21 ± 6	2020 ± 0.4
	100	<20 ± 2.1	<20 ± 0.7	<20 ± 3.5			<20	<20 ± 2.2	31100 ± 589
	500	<20 ± 0	<20 ± 1.4	<20 ± 4.2	<20 ± 2.3		<20	<20	49000 ± 147
Salix viminalis	10	<20 ± 0.7	<20	<20 ± 1.4	<20 ± 3.5		<20 ± 2.1	<20 ± 1.3	3080 ± 1.8
	100	<20 ± 0	<20 ± 1.5	<20 ± 1.4	<20 ± 0.6		<20	<20 ± 1.6	38500 ± 456
	500	<20 ± 0	<20 ± 0.8	<20			<20	<20	63600 ± 1560

APPENDIX 4 Table 5.7. Assessment of Seedling Health

Metal Concentration	After 24hrs	Day 4	Day 7	Day 16
10 Zn	no significant change in all species	Curling of upper leaves of *P.tricho.* all other species OK	All species appear healthy	*Pinus, A. glutinosa, Betula, P. euro* all appear healthy *A. rubra* and *incana* one tree dead other one healthy *P. tricho, S. viminalis* and *S. caprea* tree healthy but a few dried leaves
100 Zn	no significant change in all species	As above but *Salix* leaves curling, yellow and dry	*Betula, Pinus, P. euro and tricho*, and *A. glutinosa* healthy *S. caprea* and *viminalis* upper leaves wilted, lower leaves yellow *A. rubra* leaves wilting, *A. incana* severely wilted	*Betula* and one *A. incana* tree look healthy *Pinus, A. glutinosa,P. tricho* and *P. euro* average number of green leaves *Alnus rubra, Salix caprea* and *Salix viminalis* all leaves are dead
500 Zn	no significant change in all species	*Pop Tichocarpa* leaves black/brown. *Salix* leaves yellowed and curled	*Betula* and *Pinus* are healthy *P. euro* and *tricho* generally healthy some brown leisions *Salix viminalis* and *caprea* severely wilted *Alnus incana* leaves wilted, *Alnus glutinosa* and *rubra* severely wilted	Leaves on all species except *Betula* are dried and dead. 50% of *Betula* leaves are green (upper half of tree)

Metal Concentration	After 24hrs	Day 4	Day 7	Day 16
10Cu	upper leaves of *Salix* wilting, all other species ok	*Salix* leaves of both species yellowed and curled. *Pop Tricho* leaves dried,shriveled and curled	*Betula, Pinus* and *P. euro* were healthy *Salix viminalis* and *caprea, P. tricho, Alnus incana, glutinosa* and *rubra* wilted leaves	*Betula, A. incana* and *Rubra, Pinus, P. Euro* and *P. tricho* showing minor ill effects *Salix viminalis* and *caprea* all original leaves are dead but new green leaves growing *Alnus glutinosa* all leaves are dead
100Cu	upper leaves of *Salix* wilting, all other species OK	Leaves of *P.Trichocarpa, S.viminalis and S.caprea* are yellowed, shrivled. *P.Euro* no significant effect	*P. euro* and *tricho* 50:50 of dead to green leaves *Salix viminalis* and *caprea* severly wilted *Betula* wilted , *Pinus* limp upper stem *Alnus rubra* wilted, *Alnus incana* green soft leaves	*Betula, P. euro* and *P. tricho* and *Salix caprea* have a few remaining green leaves *Alnus incana* and *Salix viminalis* a few new green leaves to top of plant *Alnus rubra, Alnus glutinosa, Pinus*, all leaves appear dead
500Cu	no significant change in all species	*P. euro.*black/brown spots developing on leaves *Pop tricho* all leaves brown/black *Salix viminalis.*leaves and stem turning brown *Salix caprea.* veins in leaves turning brown	*Alnus glutinosa* and *Betula* generally healthy. *Pinus* wilted upper stem. *Alnus incana* and *rubra* severly wilted *P. euro* mild wilting some brown leisions. *P. tricho* all leaves yllow, black/brown spots *Salix viminaliis* and *caprea* severely wilted	*Betula, Alnus glutinosa, Alnus incana, Pop tricho* and *P. euro* have a few remaining green leaves *Pinus, Alnus rubra, Salix viminalis* and *Salix caprea* all leaves appear dead

Metal Concentration	After 24hrs	Day 4	Day 7	Day 16
10Ni	no significant change in all species	*Salix caprea* upper third of leaves dried, *Salix viminalix* OK. *P. tricho* and *P. euro* showing signs of yellowing, *P. euro* slightly worse	*Salix viminalis* and *caprea* and *Pinus* generally healthy *Betula* yellow leaves *Alnus glutinosa* and *A. rubra* wilting, *Alnus incana* more severely wilted *P. euro* and *P. tricho* severely wilted	*Pinus* and *Alnus glutinosa* majority of leaves green *Betula, Salix viminalis* and *Salix caprea* upper leaves are green *P. tricho* 95% of leaves are dead, remaining fresh growth *Alnus rubra* and *incana* and *P. euro* all leaves dried up
100 Ni	no significant change in all species	*P. euro and tricho* species affected to similar degree as 10 treatment. *Salix viminalis* and *caprea* showing yellow and curled leaves on upper half of trees	*Pinus* healthy *Betula* leaves green but soft and limp. *Alnus glutinosa, incana* and *rubra* wilted to different degrees, *A.glutinosa* to a lesser extent, *rubra* to the worst degree. *P. euro* and *tricho* severely wilted. *Salix caprea* and *viminalis* severely wilted lower leaves yelow	*Betula, P. euro* and *P. tricho* some green leaves *Alnus rubra, incana* and *glutinosa, Salix viminalis* and *Salix caprea* all leaves dead
500Ni	no significant change in all spcies	*P. euro* and *tricho* have black/brown leaves. *Salix viminalis* and caprea species ,browning of leaf veins on lower leaves	*Betula* green and yellow leaves. *Pinus* green needleas but top of stem wilted over. *Alnus glutinosa* generally healthy *Alnus rubra* and *incana* wilted. Both *Salix* and *Populus* species severely wilted	No green foliage on all trees except *Pinus* and one *Betula*

Metal Concentration	After 24hrs	Day 4	Day 7	Day 16
10 Cd	signs of wilting in upper leaves in all species especially *Populus & Salix*	*P. euro* and *Salix viminalis* OK. *P. tricho* upper leaves brown,curling and dry. *Salix caprea* top leaves are dried and curlied, bottom leaves yellow	*Pinus, Betula* and *P. euro* were healthy *Alnus incana, glutinosa* and *rubra, Salix viminalis* and *caprea* and *P. tricho* the leaves are severly wilted	*Pinus* very healthy *Alnus rubra, Alnus glutinosa, Alnus incana, Betula, Salix viminalis Salix caprea, P. euro* and *P. tricho* have a few green leaves with some new regrowth
100 Cd	signs of wilting in upper leaves in all species especially *Populus & Salix*	*P. euro* and *P.tricho* are slightly affected, some of the uppermost leaves are dry and curled, effect worst in *tricho.* *Salix viminalis* and *caprea* upper leaves appear dead, bottom leaves are very yellow.	*Pinus* and *Betula* are healthy *P. euro, P. tricho Salix viminalis* and *caprea* very withered *Alnus incana, rubra* and *glutinosa* are all withered and dead	*P. euro* and *P. tricho* have a few green leaves All other species the leaves are dead
500 Cd	signs of wilting in upper leaves in all species especially *Populus & Salix*	*P. euro* and *P. tricho* both reasonably OK, some upper leaves dry, yellow and curled. *Salix caprea* most leaves are dry and yellow *Salix viminalis* upper leaves dead, lower leaves yellow.	*Betula* leaves are healthy *Pinus* leaves begining to yellow *P. euro* upper leaves are withered, bottom leaves are healthy. *Alnus rubra, incana* and *glutinosa* and *Salix viminalis* and *caprea* and *P. tricho* are all withered	Leaves appear dead on all species except *P. tricho* with one green leaf at the very top of the shoot Note a yellow complex was obseved on the surface of all roots

Metal Concentration	After 24hrs	Day 4	Day 7	Day 16
10Cr	signs of wilting in upper leaves in all species especially *Populus & Salix*	*P. euro* upper stem wilted over. *P. tricho* upper third of leaves dried. *Salix viminalis* very tpo tips of stem yellowed. *Salix caprea* top leaves dry and crispy.	*Betula, Pinus,Alnus rubra, glutinosa* and *incana, Salix caprea* and *viminalis* and *P. tricho* are healthy (upper leaves of *S. caprea* wilted *P. euro* leaves wilted	*Pinus, Alnus incana, rubra* and *glutinosa* and *Betula* green leaves look healthy *Salix viminalis, Salix caprea* and *P. euro* have a number of green leaves on upper stem *Pop tricho* a few new leaves on lower stem
100Cr	signs of wilting in upper leaves in all species especially *Populus & Salix*	*P tricho* and *P.euro* showing signs of browning of leaves, *tricho* slightly worse *Salix viminalis* and *caprea* all leaves are dry, curled	*Pinus, Betula, Alnus incana, rubra* and *glutinosa* leaves healthy *P. euro* and *P. tricho* generally healthy some black leisions on leaves *Salix viminalis* and *caprea* wilted and dead	*Alnus incana* and *glutinosa, Betula* and *Pinus* green leaves look healthy *P. euro* and *P. tricho* have a few green leaves at the top, tricho has slightly more green leaves *Salix viminalis* and *caprea* all old leaves are dead, new shoot from the base of stem
500Cr	signs of wilting in upper leaves in all species especially *Populus & Salix*	*P. euro* and *P.tricho* all leaves are brown and dry, green stems in *P. euro* have turned brown. *Salix caprea* and *viminalis* are curled, brown and dead	*Betula* and *Pinus* generally healthy *Alnus incana, glutinosa* and *rubra, Salix viminalis* and *caprea* leaves severely wilted *P. euro* all leaves black/brown colour, *P. tricho* wilted green leaves upper shoots still green	*Betula, Alnus incana, P. tricho P. euro* and *Salix viminalis* a few green leaves at very top *Salix caprea* new leaves on new shoots appear withered *Pinus* and *Alnus rubra* appear dead

Metal Concentration	After 24hrs	Day 4	Day 7	Day 16
10Pb	no significant change in all species	no significant change in *Populus* and *Salix* species	All species generally healthy	All species look healthy apart from one *Alnus incana* tree which is dead. A few brown leaves observed on *Salix caprea*
100Pb	no significant change in all species		*Pinus, Betula, Alnus rubra, incana* and *glutinosa*, and *P. euro* generally healthy *P. tricho* slightly wilted yellow/brown leaves *Salix caprea* upper leaves wilted, lower leaves healthy *Salix viminalis* all leaves wilted	*Alnus rubra, incana* and *glutinosa* one tree dead the other tree looks healthy *Pinus* looks very healthy *P. tricho, P. euro Betula* and *Salix caprea* have a significant number of green healthy leaves *Salix viminalis* old leaves are dead new green regrowth
500 Pb	no significant change in all species	*Salix caprea* and *viminalis* only very upper leaves wilting *P. euro* healthy, *P. tricho* upper leaves begining to wilt	*Betula* and *Pinus* generally healthy *Alnus incana, rubra* and *glutinosa* generaly healthy slight wilting worse for *incana* *P. tricho* more yellow/brown leaves than 100 treatment, *P. euro* upper leaves withered dry, lower leaves limp *Salix caprea* upper leaves withered and dead lower leaves are yellow. *Salix viminalis* all leaves withered and dead	*Betula, Alnus glutinosa, Alnus incana, P. tricho* and *P. euro* some to many green leaves *Salix caprea* new leaf growth *Pinus, Alnus rubra* and *Salix viminalis* no green leaves evident